ENDOCRINES IN DEVELOPMENT

The Developmental Biology Conference Series, 1956

HELD UNDER THE AUSPICES OF

THE NATIONAL ACADEMY OF SCIENCES
NATIONAL RESEARCH COUNCIL

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ENDOCRINES
IN DEVELOPMENT

Edited by
RAY L WATTERSON



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Preface to the Series

Development and growth have usually been studied rather piecemeal as embryology or plant physiology, or nutrition, or oncology as seriation of stages of chick embryos as cell division in fish eggs or plant root tips as growth curves of children as hormone response of plumage, as spread of a fungus as repair of a broken bone or the swelling of a diseased spleen, by observation, measurement comparison chemical alteration excision, transplantation or sheer speculation Yet, in reality all of these are merely isolated aspects of one broad continuous spectrum of phenomena varied manifestations of the same basic principles and elementary processes multiplication of organic mass (growth) diversification of that mass (differentiation) pattern formation (morphogenesis), progressive change (maturation and aging) and the repair or reproduction of patterns after disturbance (regulation and regeneration)

This unity of subject matter has received renewed emphasis in the Developmental Biology Conference Series of 1956 a record of which is now presented in ten volumes including the present report The series consisted of co ordinated and interdisciplinary conferences symposia and workshops organized under the sponsorship of the Biology Council of the Division of Biology and Agriculture, National Academy of Sciences–National Research Council with the generous financial support of governmental agencies industrial organizations and private foundations and donors (see list at end of Preface)

The Conference Series brought together experts from the fields of anatomy biochemistry biometry biophysics botany cytology embryology endocrinology genetics histology immunology microbiology neurology nutrition oncology pathology physiology radiology and zoology from the United States and abroad less for a display of most recent technical advances than for a concerted examination and evaluation of the contributions of these various specialties to the elucidation of focal issues of developmental biology Fresh orientation and new ideas could be expected to emerge from this pool of critically distilled knowledge by the intersection of formerly unrelated trends of thought or by the discovery of common cores in formerly unrelated sets of data Nearly three hundred

American and fifty four foreign scientists (from nineteen countries) joined in this task

To serve the outlined objective, the meetings had to be ruled by the key words *perspective* and *relevance* All participants were admonished to present only such itemized information, conclusions, demonstrations, criticisms, illustrations, questions and quotations as promised to throw light on the *issue* under discussion that is to confine themselves to com ments of "strategic" or 'catalytic" pertinence, not merely adding to the bulk of information, but contributing to clarification order, harmoniza tion, and comprehensibility Pertinent comments however, were wel comed regardless of whether they referred to data so new as to be still largely unknown so old as to have been widely forgotten, so specialized or technical as to have received limited currency among 'outsiders', so theoretical as to have escaped the practitioners, or so ' self evident" as to have evaded critical scrutiny Each participant was expected to draw from his store of special knowledge points that might help correct mis interpretations, indicate the feasibility of new approaches and above all reveal existing gaps of knowledge and understanding

It is evident that a group exercise of this complexion with free give and take could not possibly ' cover the ground ' in any of the selected topics within the given time limits and without sacrificing spontaneity informality and depth of penetration Often a few key issues, profoundly analyzed and critically elucidated proved far more enlightening than a hurried bird s eye view of a large field In cases in which workers from different disciplines were only vaguely acquainted with one another s stock in trade and vocabulary, the time and effort spent describing even elementary facts in order to provide a common ground for communication proved very worthwhile indeed In other instances where the facts were familiar but their interpretation was controversial it seemed preferable to let argumentation take precedence over the recital of facts

The foregoing remarks are intended to explain the discursive nature of these volumes In conferences which combine basic and clinical inter ests technical and theoretical approaches, molecular and organismic con cepts botanical and zoological subjects biochemical and morphological aspects it is imperative to place the reconciliation and synthesis of view points *above all other considerations*

In line with this general precept each conference chairman was to open his meeting with a brief keynote address, staking out the major problems for discussion By phrasing questions rather than stating theses he was to set the stage for free though not necessarily unpremeditated

participation Most of the conferences were closed meetings with attendance confined to the invited panel members In a few cases auditors were admitted Only the symposia at Brown University, which were co-sponsored by the International Union of Biological Sciences were open to the general public

For the purpose of publication, an experienced scientist familiar with the subject matter was appointed as official reporter and editor for each conference, to attend all sessions without taking part in the discussions From the sound tape recordings of the proceedings and his or her own notes each editor then produced a condensed version of the conference These accounts constitute the substance of this series of publications

The individual reports vary greatly in form depending on the topic and organization of each conference as well as on the personal predilections of the editor Only in one instance has the dialogue style been kept, and even so only after considerable pruning In other cases an entire conference has been reported as a third person account reordering the text rather liberally into a logical sequence by combining related fragments in this process of synthesis an editor assumed the full prerogatives of an author Most of the reports, however, range somewhere between these two extremes abstracting the major comments of the various participants without obliterating their identity yet resorting to verbal quotations infrequently or not at all Some participants furnished their own rewritten versions of the factual presentations and these were in most cases inserted in the text as such In all cases the participants were given an opportunity to check their respective contributions either in the original transcript or later in the condensed and revised text

The lack of uniformity reflects the informal spirit of the meetings and accents the main theme of the Conference Series that developmental biology is currently in a state of flux fitting no rigid mold and shaping its own course as it gains momentum by the growth and confluence of its many tributaries It is hoped that the publication of this series will add to that momentum as did the conferences themselves

To each of the participants but above all to the chairmen and editors we owe a deep debt of gratitude To the following donors of funds we reiterate our appreciation for generous assistance Atomic Energy Commission U S Departments of the Air Force Army and Navy (Medical Services) Office of Naval Research Fulbright Fellowship Program National Institutes of Health National Science Foundation International Union of Biological Sciences American Cyanamid Company Diamond Alkali Company Merck and Company Inc Chas Pfizer and Company

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PAUL WEISS

NEW YORK CITY
May 1958

SYMPOSIUM ON

Endocrines in Development

HELD SEPTEMBER 11–13 1956

AT

SHELTER ISLAND NEW YORK

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Baltimore Maryland

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Contents

List of Illustrations

I
Introduction

The conference was called to order by Dr Weiss, who emphasized that the sessions would deal with various aspects of the relation between endocrines and development He pointed out that this is a two way street Endocrine glands undergo development and as they develop they influence other organs including other endocrine glands Development may be considered in a broad sense as the large tidal wave of change that leads from conception to death not broken up by the ripples of cyclic physiologic activities Viewed in this light the study of endocrines in development extends beyond the embryonic period into later stages including maturation puberty metamorphosis regeneration pathologic changes and metaplasia Weiss introduced Dr Willier chairman of the conference who immediately reviewed the history and the highlights of the subject under discussion

Dr Willier stated that he began his graduate work at a time when endocrinology was in its infancy He first encountered the term hormone as a student of A J Carlson and F R Lillie at the University of Chicago He participated in the analysis of the freemartin effect He noted that the goal of the present conference is to exchange knowledge and understanding of the role of endocrines in the ontogenetic process He suggested that the subject under discussion should be called ' Genetic Endocrinology Weiss (1950) coined the title Genetic Neurology for a conference devoted to problems of development growth and regeneration of the nervous system Genetic endocrinology might be to paraphrase Dr Weiss a discipline encompassing all those processes that lead up to the mature state of the endocrine system Key issues concerning the part that hormones play in the ontogenetic process should be presented These should be analyzed and elucidated critically from new angles Gaps in knowledge and understanding should be emphasized An attempt should be made to develop a common scheme or generalization into which seemingly unrelated facts can be fitted New ideas should be engendered Every effort should be made to evolve a

4 The receptor responds according to its intrinsic potentialities which it acquires during the course of early development The receptor develops reaction capacities before hormones are poured into the circulation This concept is implied but not specifically stated in early papers Lillie stated (1917 p 419) "In the case of the free martin we do not find that male hormones cause the development of any structure which is not represented embryologically in the normal female, the hormones act in this case merely by inhibition or stimulation of normal embryonic rudiments The concept as we now understand it is clearly implied here The following quotation is from a later paper by Lillie (1929, p 521) Goldschmidt also (1927) compares his organ forming substances to hormones So far as embryonic segregation i e the origin of specific potencies is concerned the analogy is entirely inappropriate because as I pointed out with reference to the sex hormones (Lillie 1927*b*) the action of hormones presupposes specific potency of the substrate The hormone does not act by creation of segregates but only by stimulation or inhibition of existing substrates Clearly the concept of selective response of the receptor is inherent in these statements quoted from Lillie s text

5 Hormones act synergistically to produce their effects This concept can be traced back to a paper of Smith (1933) or even earlier if one reads between the lines Smith was the first to show that hormones produced by the anterior pituitary and by the thyroid have a synergistic action in promoting the growth of the rat This concept has been confirmed by investigations at the University of California utilizing purified hormones such as thyroxin and growth hormone (Simpson Asling and Evans 1950 Geschwind and Li 1955) Thus an interlocking action of hormones is involved in regulating growth and development

6 A reciprocal influence is established between endocrine glands This has been emphasized by Moore and Price (1932) Their discovery is known to everyone working in this field and certainly deserves recognition here

7 Vertebrate and insect hormones have been isolated purified and/or synthesized First the synthesis of adrenalin should be mentioned and next the synthesis of thyroxin Steroid hormones both sex hormones and adrenocortical hormones were synthesized later Next should be mentioned the greatest triumph of all because it dealt with proteins namely the determination of the structure of three species of insulin molecules —those of cattle sheep and hogs—by Sanger and his collaborators in recent years (Sanger and Tuppy 1951*a* *b* Sanger and Thompson 1953*a* *b* Sanger Smith and Kitai 1954) Almost equally remarkable

coherent and consistent picture of the role of the hormones in the growth and development of the organism.

Genetic endocrinology is a relatively young science, dating back to the investigations of Gudernatsch in 1912 and Adler in 1914 The work of Gudernatsch demonstrated that feeding thyroid to frog tadpoles initiates precocious metamorphosis within a few days Two years later Adler reported in his significant paper that destruction of the anterior pituitary in frog tadpoles results in profound changes in the thyroid gland (growth retardation, production of a small amount of abnormal colloid etc) and likewise in the failure of the tadpoles to undergo metamorphosis These pioneer investigations, although lacking in finish and polish, nevertheless opened the field to more critical and refined modes of exploration of the role of hormones in development Progress was relatively slow in this subject for four decades Within this period there are certain highlights in the history of developmental endocrinology, a few of which follow

1 The functional relationships between the anterior pituitary, the thyroid the adrenals and the gonads are established during the course of development of the embryo At first the *functional dependence* of the various glands was stressed Thus it was realized that the thyroid is dependent upon the anterior pituitary in some way Gradually the concept of the *functional interdependence* of the various glands emerged an interdependence of anterior pituitary and thyroid, of anterior pituitary and adrenal It is curious that the emphasis was placed at first upon the thyroid pituitary relation rather than upon the adrenal pituitary relation because Smith *(1920)* showed that when he removed the pituitary from tadpoles the adrenal as well as the thyroid is affected

2 Sex hormones act during the embryonic development of sex organs This was demonstrated by the studies of Lillie *(1916 1917)* on the freemartin One facet of this analysis constituted part of Willier s doctoral thesis Everyone is familiar with the impact that this discovery had upon investigations of the role of sex hormones in sex differentiation

3 The endocrine receptor or end organ serves as a target for hormone action The word target should always be used in quotes The origin of this term is uncertain it was probably laboratory jargon initially There can be no target in the usual sense because a target is something that is shot at Hormones are not shooting at anything they are in circulation they are picked up selectively The terms receptor and 'end organ' were borrowed from nerve physiology of the English school

II

Ontogeny of Selected Endocrine Glands
The Thyroid

DR WILLIER introduced the topic by outlining the development of the thyroid This gland arises from the floor of the pharynx A small bud is cut off which becomes circular in outline This circular body constricts into two by some process of morphogenesis which is not understood resulting in formation of two circular bodies one right and one left. Thus the thyroid epithelium exists in the form of two sacs WEISS inquired whether any abnormality is known that affects this dichotomy He called attention to the similarity of the dichotomy that occurs during lung development and stated that they were attempting with some success to block the latter experimentally He emphasized that application of the classical methods of embryology to the problem of the factors involved in producing the dichotomy of the thyroid rudiment would probably be productive of results BURNS remarked that he could recall no case where this dichotomy is completely arrested He did point out that the finished organ is never completely subdivided the two lobes remain more or less connected He recalled that a median thyroid can persist in its own right to give rise to a thyroid mass which may even be imbedded in the base of the tongue quite close to its original point of origin

WILLIER continued by stating that this epithelium breaks up into a series of interlocking cords surrounded by a connective tissue capsule Between these epithelial cords are large spaces resembling sinusoids filled with blood This blood is received from a branch of the internal carotid artery (see Willier 1955 Fig 208) The sinusoids are especially conspicuous at the time when the interlacing cords begin to form follicles and colloid begins to appear in them. A critical stage in the development of the thyroid with respect to the formation of sinusoids and their association with follicles occurs between the tenth and eleventh days of incubation in the case of the chick

Willier s use of the term epithelial cords was questioned by GAIL

was the determination of the structure of hog beta corticotrophin by Bell in 1954 and by Howard *et al* in 1955 and of sheep alpha corticotrophin by Li *et al*, of the University of California, in 1955 Almost simultaneously appeared the work of Du Vigneaud (1950) and his associates on oxytocin and vasopressin which are small peptide hormones of the posterior pituitary Thus the constitution of many hormones is now well known Yet how little is known about the crucial reactions which these hormones release in the terminal receptors! Discovery of the chemical constitution of hormones will surely have an impact on studies concerned with the mechanism of action of hormones on receptors The receptor picks up the hormone molecule selectively, somewhat as a television set picks up frequencies selectively There is some kind of matching between hormone molecules and something in the receptor They fit together somehow, and knowledge of the chemical constitution of hormones may aid in understanding how hormones are picked up selectively and how hormones produce changes in receptors

This would argue against the presence of any thyrotrophic hormone in the plasma of the clot

Weiss then inquired whether mitotic division occurs without concurrent division of the cytoplasm Gaillard answered in the affirmative Weiss then called attention to the fact that there had long been a suspicion that the neural tube was a syncytial organ but that more penetrating analyses (Sauer 1935*a* *b* Watterson *et al* 1956) revealed it to be truly cellular Cell boundaries are simply very difficult to see He also remarked that the electron microscope reveals cell boundaries in embryonic stages where they were thought to be lacking He questioned whether it is certain that distinct cells are lacking in thyroids of embryos Gaillard answered that they cannot be seen at first with the *highest magnification of the light microscope, even if the preparations* are stained with Hellblau which usually reveals cell boundaries very nicely One or 2 days later they can be seen Weiss commented that he was emphasizing this point because an extremely interesting principle of morphogenesis would be involved This would be one of the few cases of a nucleated tube growing out into a linear structure similar to the hyphae of fungi Weiss considered the problem to be worthy of further investigation

Willier continued by stating that there is considerable evidence that the primordium of the thyroid can pick up radioactive iodine selectively before there are any follicles indicating that specific cell strains are already *involved In other words the cells are already fixed as specifically* thyroid cells He called attention to a recent paper, the reference to which he could not recall where someone had introduced radioactive iodine into a human mother following which the thyroid of the embryo had picked up the radioactive iodine selectively In this case it had gone through the placenta Gaillard mentioned that he had some radio autographs showing the addition of one eightieth of a millicurie of I^{131} to cultures selective absorption of I^{131} occurs approximately 1 day before the first sign of colloid formation occurs in vitro i e before the colloid can be distinguished histologically

Weiss then inquired about the thyroid tissue which grows out from the explant *in vitro* He was *thinking of the work of Carpenter (1942)* and others He asked whether distinct cells were visible in the epithelial outgrowth Gaillard answered that she had cultivated whole organs and had not obtained an epithelial outgrowth However, Demuth (1933) and Ebeling (1924) grew fragments of older thyroid glands on cover glasses years ago and obtained epithelial membranes with well bordered cells

LARD He inquired whether individual thyroid cells could actually be distinguished from one another In his work on thyroid development he never sees any sign of borders between cells at this time, he sees only syncytial or symplasmic cords containing many nuclei Cell boundaries appear quite suddenly a little later In response to these questions, WILLIER asked whether it was not true that the cell surface was not very well defined in many animal tissues particularly during embryonic development GAILLARD then commented on his observations on thyroid development as follows

In thyroid glands cultured in the absence of the anterior pituitary and thus in the absence of anterior pituitary hormones, unless the latter are present in very low concentration in the human or cock plasma which is used, colloid droplets always arise quite close to the nuclei juxtanuclear, within symplasmic cords In the chick embryo the droplets can be found at any place in the thyroid gland in between nuclei When the thyroid gland from an 8 day chick embryo is cultivated, further development of the tissue structure occurs rather slowly This means that the symplasmic stage sometimes last for 2 or 3 days During this period hundreds of small colloid droplets develop in close relation to the nuclei they then increase in volume and sometimes push aside neighboring nuclei Gradually a number of nuclei become arranged around a big colloid droplet, and it is about this time that cell borders develop microscopically Follicle development is retarded for 1 or 2 days Follicle *formation begins but follicles with high columnar epithelium are never* formed All cells remain cuboidal Even in the presence of TSH (thyroid stimulating hormone) fully developed follicles never form in vitro, although an increased amount of colloid is observed Colloid formation and the arrangement of cells into follicles are therefore to be considered as dissociable processes The cords mentioned by Willier do not often develop during cultivation in vitro, although they are seen occasionally in glands cultivated in the presence of TSH In these cases the spaces between the cords resemble sinuses they are filled with a substance which is slightly eosinophilic and with some erythrocytes and mononucleate cells

WILLIER stated that some development independent of stimulation from some other endocrine gland is a characteristic feature of all endocrine glands but that development will continue only to a certain point in the absence of such stimulation BURNS said that this is certainly true in completely hypophysectomized amphibian larvae Even rudimentary follicles may be present as well as small amounts of colloid (Smith 1920) Then development stops without further stimulation

This would argue against the presence of any thyrotrophic hormone in the plasma of the clot

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WEISS asked to what extent it is possible to dissociate the effect of TSH on differentiation or rate of development of the gland from its effect on the production of a product, namely, colloid GAILLARD answered that TSH causes only increased production of colloid and has no effect on the development of thyroid structure WILLIER commented that in the experiments of Fugo (1940), who hypophysectomized chick embryos by extirpation of the forebrain, the development of the thyroid is slowed by the eleventh day of incubation, suggesting that the speed of development is reduced in the absence of thyrotrophic hormone GAILLARD stated that Fugo's results caused him to expect that TSH would have an influence on the formation of follicles when added to his thyroid cultures, but there is no such effect WEISS then inquired whether TSH has any action on the discharge of colloid from thyroid cells GAILLARD called attention to the experiments of Gonzales (1956) who found that thyroxin, mono, and diiodotyrosine, as well as some unknown iodine labeled products are present in explanted thyroids but not in the culture medium Such experiments indicated that no discharge of the products had occurred By contrast, corticosteroids produced by explants of the adrenal cortex of young rats are released into the culture medium MARTINOVITCH questioned the results obtained by Gonzales He stated that Pavlović, Roche and Michel were studying the function of explanted thyroid glands of infantile rats with the aid of I^{131}, electrophoresis, and paper chromatography and that they could demonstrate the presence of mono and diiodotyrosine in the culture medium

WEISS then presented a general scheme of problematic interactions and feedback mechanisms that should be kept in mind in an analysis of any relationships as complex as those between the pituitary and the thyroid (Fig 1) The growth and differentiation of the pituitary gland lead to formation of a product (TSH) which is then discharged The discharged TSH acts on a structure in the process of growth and differentiation namely the thyroid gland The latter structure in turn is producing certain products one of which is thyroxin which may or may not be discharged Does TSH affect growth of the thyroid gland (Fig 1, arrow *1*) differentiation of the thyroid (arrow *2*) formation of the product of the thyroid (arrow *3*), or discharge of the product (arrow *4*)? Does the product of the thyroid affect growth (arrow *5*) and/or differentiation (arrow *6*) of the thyroid while it is still inside the thyroid gland or does it affect growth (arrow *7*) and/or differentiation (arrow *8*) of the thyroid cells only after it is discharged from the thyroid? Does the discharged product of the thyroid gland also affect the

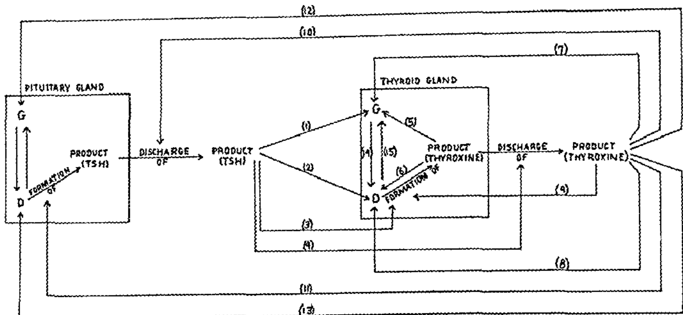


FIG 1 —Problematic interactions and feedback mechanisms between the pituitary gland (source of trophic hormone TSH) and the thyroid gland (source of thyroxin) G = growth D = differentiation (Weiss original)

production of the product (arrow *9*)? Does the discharged product of the thyroid affect only the discharge of TSH by the pituitary gland (arrow *10*), or does it also affect the production of TSH by the pituitary (arrow *11*)? Does discharged thyroxin have any effect on the growth (arrow *12*) and/or differentiation (arrow *13*) of the pituitary gland? Perhaps within the thyroid there is some sort of antagonistic relation between growth and differentiation such that, when growth is stimulated, differentiation is inhibited (arrow *14*) or vice versa (arrow *15*) Perhaps the mere act of discharge of the thyroid product stimulates the thyroid gland to produce and/or to accumulate more of its product Such a diagram serves to emphasize the fact that merely stating that TSH produced by the pituitary gland stimulates thyroid activity and that thyroxin produced by the thyroid gland inhibits pituitary activity is an oversimplification of the actual situation

TONUTTI recalled that Rawson and Money (1949) were concerned with some of the questions raised by Weiss and stated that Fabbrini (1955*a*) working in his laboratory studied the influence of thyroxin and triiodothyronine on the growth promoting action of a given amount of TSH on the thyroid gland of hypophysectomized rats where no release of TSH is possible The growth promoting action of TSH was found to be diminished SEGAL pointed out that the administration of a given hormone causes hypofunction and atrophy of the corresponding endocrine gland It certainly does not cause growth of the gland that normally secretes the administered hormone This is true for thyroxin administration and its effect on the thyroid sex hormone administration and its effect on the gonads or corticoid administration and its effect on the adrenals This principle also holds when there is an imbalance in the production of endogenous hormones In cases of hyperfunction of the adrenal cortex with an overproduction of adrenal androgens gonadal hypofunction results Segal believes that the possibility that a hormone can exert an effect on the organ producing it still remains open

MOOG stated that it has been shown experimentally that administration of cortisone causes no additional reduction in the size of the adrenals of hypophysectomized rats SEGAL agreed but pointed out that by that time the adrenals are down to the minimal size, which is independent of the trophic action of ACTH *He suggested that before this* minimal level is reached the direct action of a steroid hormone on the adrenal cortex must still be considered a possibility For example the direct inhibitory influence of steroids on one or the other component of the embryonic gonad is clearly shown by experiments involving administration of steroids to amphibian larvae sex reversal is the con

sequence This occurs in the presence or in the absence of the hypophysis (Chang and Witschi, 1955a) TONUTTI mentioned that in their unpublished studies of nuclear size in the adrenal cortex of hypophysectomized animals they found no additional decrease in size after administration of cortisone

WILLIER then called attention to experiments carried out by one of his former students (Dossel, 1954, 1957), who isolated the thyroid sac of chick embryos and transplanted it to some other location on the body of another embryo In certain experiments it was placed in the head close to the host pituitary, in other experiments it was placed posteriorly, near the base of the allantoic stalk When transplanted to the head, together with its surrounding mesenchyme it forms a mass of typical thyroid tissue But if the thyroid sac is transplanted free from its surrounding mesenchyme (removed either by enzyme treatment or by dissection) no growth of the graft occurs Thus the local mesenchyme of the thyroid rudiment seems to have a highly specific action, foreign mesenchyme of the head will not substitute for it in such a way as to enable the thyroid rudiment to undergo further development Similarly, the foreign mesenchyme of the tail cannot substitute for the specific mesenchyme of the thyroid gland Thus the mesenchyme of the thyroid gland appears to play an important role in the development of the thyroid but the nature of this role remains unknown GAILLARD recalled the very similar role of specific mesenchyme in the development of other structures as demonstrated by Grobstein (1953) BURNS mentioned that the particular mesenchyme in the vicinity of the bulbo urethral glands can be recognized before the gland itself has developed very far A large area of condensation appears in the mesenchyme lateral to the urogenital sinus at the point where the bulbo urethral gland is to appear The gland itself is represented at this time by a small bud on the surface of the sinus Probably the mesenchymal capsule of other glands plays a much more significant role in their development than has previously been recognized WEISS reminded the conference that he had long been an advocate of the notion of differentiation of the mesenchyme If the pre mesenchyme of the limb bud is isolated and is dissociated into its individual cells which are allowed to reaggregate at random in vitro cartilage of the limb type is formed If the mesenchyme around the eye is similarly isolated and dissociated into individual cells before there is any cartilage or pre cartilage a plate of sclera cartilage forms following their reaggregation in vitro and this type of cartilage is quite different from that

production of the product (arrow *9*)? Does the discharged product of the thyroid affect only the discharge of TSH by the pituitary gland (arrow *10*), or does it also affect the production of TSH by the pituitary (arrow *11*)? Does discharged thyroxin have any effect on the growth (arrow *12*) and/or differentiation (arrow *13*) of the pituitary gland? Perhaps within the thyroid there is some sort of antagonistic relation between growth and differentiation such that, when growth is stimulated, differentiation is inhibited (arrow *14*) or vice versa (arrow *15*) Perhaps the mere act of discharge of the thyroid product stimulates the thyroid gland to produce and/or to accumulate more of its product Such a diagram serves to emphasize the fact that merely stating that TSH produced by the pituitary gland stimulates thyroid activity and that thyroxin produced by the thyroid gland inhibits pituitary activity is an oversimplification of the actual situation

TONUTTI recalled that Rawson and Money (1949) were concerned with some of the questions raised by Weiss and stated that Fabbrini (1955*a*) working in his laboratory, studied the influence of thyroxin and triiodothyronine on the growth promoting action of a given amount of TSH on the thyroid gland of hypophysectomized rats where no release of TSH is possible The growth promoting action of TSH was found to be diminished SEGAL pointed out that the administration of a given hormone causes hypofunction and atrophy of the corresponding endocrine gland It certainly does not cause growth of the gland that normally secretes the administered hormone This is true for thyroxin administration and its effect on the thyroid sex hormone administration and its effect on the gonads or corticoid administration and its effect on the adrenals This principle also holds when there is an imbalance in the production of endogenous hormones In cases of hyperfunction of the adrenal cortex with an overproduction of adrenal androgens gonadal hypofunction results Segal believes that the possibility that a hormone can exert an effect on the organ producing it still remains open

MOOG stated that it has been shown experimentally that administration of cortisone causes no additional reduction in the size of the adrenals of hypophysectomized rats SEGAL agreed but pointed out that by that time the adrenals are down to the minimal size which is independent of the trophic action of ACTH He suggested that before this minimal level is reached the direct action of a steroid hormone on the adrenal cortex must still be considered a possibility For example, the direct inhibitory influence of steroids on one or the other component of the embryonic gonad is clearly shown by experiments involving administration of steroids to amphibian larvae sex reversal is the con

III

Ontogeny of Selected Endocrine Glands The Adrenals

The development of a second endocrine gland the adrenals was then reviewed by DR WILLIER He recalled that this is a composite organ composed of cortex and medulla and that these two components have separate origins The cortex arises from a series of cords that invaginate from the coelomic epithelium just anterior to the site of the embryonic gonads The medullary or chromaffin cells arise from the sympathoblasts, which are capable of forming either sympathetic neurons or chromaffin cells The sympathoblasts are presumably derivatives of the neural crest Willier (1930) demonstrated that the cortex can arise quite independently of the sympathetic component At least it is possible to obtain chorio allantoic grafts composed of typical cortical cords in the complete absence of chromaffin cells Such grafts are isolated originally from donors in the head process stage He stressed the fact that only those sympathoblasts which come into intimate contact with cortical cells give the chromaffin reaction this reaction indicates the presence of adrenalin or noradrenalin the precursor of adrenalin He stated that the sympathoblasts of mammalian embryos migrate through the cortex as little nests of cells (Flint 1900), which develop a characteristic vascular pattern resembling the glomeruli of the kidney (see Willier 1955 FIG 209, *B*) After onset of this migration the sympathoblasts not only show the chromaffin reaction but also react positively to physiologic tests for the presence of adrenalin However, if contact of sympathoblasts with cortex is essential for the differentiation of these cells into medullary cells of the adrenal it is difficult to understand why other sympathoblasts which do not make this contact are able to differentiate into the organs of Zuckerkandl, i e into the chromaffin bodies located up and down the vertebral column These are known to contain adrenalin or noradrenalin the latter predominating in the fetus the former in the adult

WILLIER also emphasized the specific nature of the circulatory pat

of a limb Thus the mesenchyme is clearly differentiating in each case in accordance with the organ territory from which it is taken

WILLIER then called attention to still another striking example of the differentiation of the mesenchyme provided by Cairns and Saunders (1954) A piece of mesenchyme taken from the prospective thigh region of a leg bud of a chick embryo and placed in the prospective shoulder region of a wing bud induces formation of thigh type feathers on the wing Thus the mesenchyme of the leg bud is already specified at the time of transplantation, not only as leg mesoderm but as mesoderm of a specific region of the leg He suggested that there is a great diversity of problems which could be analyzed to determine the role of specific mesenchyme in the development of endocrine glands and endocrine receptors

SEGAL added still another example of this phenomenon In the development of the adrenal of selachians, Chieffi (1952) described a mass of cells that he believed to represent a proliferation of the coelomic epithelium or of the dorsal root of the mesentery These cells give rise to the adrenal cortex They could just as well be interpreted as a condensation of the mesenchymal tissue in this region Thus they could be interpreted as a specific part of the mesenchyme which plays an important role in the development of one of the endocrine glands WEISS stated that actually, the entire mesodermal component of the limb bud of the tadpole comes from the coelomic epithelium The peritoneum becomes loosened in a localized area and cells migrate out to form the solid mass of mesenchyme which constitutes the mesodermal core of the limb bud (Taylor, 1943 1944)

areas which are, in fact continuous with each other if observed early enough in development Witschi s Figure 85 (1956) shows a stage just after hatching in *Rana sylvatica* larvae, the lateral mesodermal plates have fused in the mid line The right and left cardinal veins are still lateral in position but they now begin to migrate medially, to fuse ventral to the dorsal aorta to form the single caval vein In doing so, the right and left cardinal veins cut beneath the intermediate meso derm eparating the latter from the lateral mesoderm The right and left masses of intermediate mesoderm then migrate medially until they fuse in the dor al mid line between the dorsal aorta and the newly formed caval vein, to form the first definitive adrenal mass From this the right and left adrenals eventually start their ventrolateral migra tion along the venous lacunae bordering on the mesonephric bodies (Witschi 1956 Fig 90) The cortical elements are then joined by sympathoblasts, which leave the sympathetic ganglia These nests of sympathoblasts assume chromaffin characteristics as they leave the ganglia and give rise to the adrenal medulla Thus there really is not much difference in the two views concerning the origin of the adrenal cortex The one states that the cortical rudiment arises from the coelomic epithelium the other that the cortical rudiment originates from the intermediate mesoderm Both sources are actually continuous at earlier stages than those usually examined

Segal called attention to one other point The interpretation pre sented above emphasizes that the adrenal cortex and the medullary component of the gonads actually have a common origin from the intermediate mesoderm Hence the functional similarities between the two become reduced to an embryological basis It thus becomes possible to speak of the steroid glands—the adrenal cortex and the gonadal medulla both originate from a common primordium

Gaillard then commented on some unpublished observations of Dr Schaberg made in his laboratory on the functional activity of adrenal glands of the 5 day rat grown in vitro He cultivates the outer portion of the cortex with the adjacent connective tissue After a few days the connective ti sue capsule grows to surround the whole explant and all spongiocyte type cells disappear Only a very regularly distributed glomerulosa type cell develops In confronted cultures where this type of adrenal cortical explant is combined with fresh anterior pituitary fragments taken from rats of the same age fairly large vacuolated cells develop inside the adrenal explant these exhibit all the cytologic characteristics of the stimulated spongiocyte type cell But if the an terior hypophysis tissue is cultivated for 6 days prior to combining

tern of the adrenals, which is present by the time the glands are known to be producing various cortisones The development of the characteristic palisade structure of the adrenals is not dependent upon the development of this specific circulatory pattern MARTINOVITCH showed a photomicrograph of a section of a cultured adrenal gland taken from an infantile rat, the typical palisade structure had developed in vitro in the absence of blood vessels WEISS suggested that the palisading of the adrenal cells, as well as the special orientation of the capillaries, probably represents a response to some more basic structure of the ground substance, instead of one being the cause of the other KOLLROS referred briefly to his work (1949), which showed that grafted heads of tadpoles develop an arrangement of the major blood vessels at the surface of the brain, as well as of the capillaries, which is quite different from normal brains This would seem to imply that the basic structure of the ground substance of the brain is not the only factor involved in the development of specific vascular patterns For example, there are fewer radially arranged penetrating vessels in the midbrain portion of the graft than in the corresponding region of control embryos

TONUTTI inquired whether anyone participating in the conference was familiar with the so called second proliferation of the adrenocortical tissue In the human embryo the first proliferation from the coelomic epithelium forms the fetal cortex whereas the second proliferation occurring after birth establishes the definitive adrenal cortex at the surface of the fetal cortex (Keene and Hewer, 1927 Uotila, 1940) This caused BURNS to comment on the parallel between the development of the adrenals and that of the gonads They are located very close together in early stages of development and they both show two generations of proliferation, resulting in the case of the gonad in the formation of two generations of sex cords The development of these two structures becomes increasingly parallel the more it is pursued

WILLIER then called attention to the fact that Witschi (1956) presents a different account of the origin of the adrenals from the one given above SEGAL presented Witschi s point of view and attempted to show that there is actually very little difference in the two interpretations He referred to Figure 54 in Witschi's textbook, which shows the intermediate mesoderm of *Hyla crucifer* located between the somite mesoderm and the lateral mesoderm The region of the lateral plate that will later become the splanchnic mesodermal plate is in direct contact with the intermediate mesoderm If this relationship is kept in mind it is clear that the question concerning the origin of the adrenal cortex is simply whether it arises from one or the other of the two mesodermal

with the anterior pituitaries in tissue culture via nerve fibers GAILLARD answered that no such fiber connections are established in the cultures

SCHNEIDERMAN then stated that, although neurosecretory substances are tremendously important in the co-ordination of endocrine activities, investigators have succeeded in getting isolated neurosecretory materials to work in only a few instances For example, 50 silkworm brains can be ground up and extracted, and the extract can be tested for neuro secretory activity in an appropriate insect test system But there is no activity However, if a single living brain is implanted into the same test system, tremendous neurosecretory activity is obtained Only Guillemin (1957) and Guillemin *et al* (1955 1956) succeeded in get ting active extracts from the various hypothalamic nuclei of mammals TONUTTI mentioned that he had made such extracts and that he could also isolate active substances by chromatographic techniques SCHNEI DERMAN then inquired whether they are proteinaceous materials TONUTTI answered that they are polypeptides MOOG added that Saffran Schally, and Benfey (1955) had some evidence that the corticotrophin releasing factor is a peptide WILLIER then asked whether the posterior pituitary has the same effect as the hypothalamus he would expect this since neurosecretory nerves from the hypothalamus terminate in the neural lobe of the pituitary GAILLARD answered that this has not been tested

MARTINOVITCH then pointed out that spongiocyte-like cells can ap pear in cultures of rat adrenal glands in the absence of both the pituitary gland and ACTH They either form as islands of cells in the mass of undifferentiated cortical tissue or are scattered through it individually or in pairs Similar cells are found in adrenal glands of hypophysecto mized grafted rats and in the adrenals of animals which are incompletely hypophysectomized (Martinovitch 1955)

WILLIER inquired whether Gaillard had made any histochemical or cytochemical tests on adrenal gland cultured in vitro GAILLARD re plied that spongiocytes give a positive reaction to Sudan black WILLIER then asked whether the reactions are any different when the anterior pituitary is associated with the adrenals in vitro GAILLARD answered that the typical reactions are found as soon as the spongiocytes have developed under the influence of the anterior hypophysis SEGAL asked for a description of the normal adrenal gland at this stage GAILLARD stated that they show a beautiful stratification with a zona glomerulosa and a zona fasciculata But as soon as they are explanted all fascicu lata type cells disappear Most of them die If fresh anterior pituitary or ACTH or cultured pituitary tissue plus hypothalamus is added to the adrenal cultures after the fasciculata cells have disappeared, this

fragments of this gland with the explants of adrenal cortex, it has no effect on the latter Adrenal cortical explants cultivated in the presence of ACTH exhibit the same kind of changes as under the influence of fresh anterior pituitary tissue In fact, Dr Schaberg studied the effects of different concentrations of ACTH (from 10 IU/cc to 1/1 000 000 IU/cc) An optimal effect is obtained with 2 IU/cc to 1/50 IU/cc Considering the fact that fresh anterior hypophysis explants and ACTH give analogous effects, it seems likely that the fresh hypophysis tissue releases ACTH into the culture medium

In order to study the effect in more detail it was decided not to try to estimate the amount of ACTH in the medium because this hormone is known to be a rather unstable compound in blood plasma at 37° C Instead, it was decided to try to estimate the amount of corticosterone in the medium with the help of the Saffran test (Saffran and Schally 1955) Transformation of glomerulosa cells into spongiocyte cells always coincides with an increased amount of corticosteroids in the medium Conversely no increase in corticosteroids is found when formation of spongiocytes does not occur

As described above, precultivation of anterior hypophysis tissues leads to a loss of effect on adrenal explants This lack of effect could be overcome by the addition of hypothalamic tissue taken from the region of the median eminence This discovery reveals that the cultivated anterior hypophysis tissue is still capable of releasing ACTH but that it lacks some activator which normally originates in the hypothalamus SCHNEIDERMAN inquired whether it was necessary to have cellular continuity between the hypothalamic tissue and the cultured pituitary in order to get this effect GAILLARD stated that such continuity is not necessary SCHNEIDERMAN pointed out that this is exceedingly interesting because, to his knowledge there have been few demonstrations of an action of neurosecretory cells in this manner when they are separated from their normal end organs GAILLARD commented that in the present experiments hypothalamus tissue and anterior hypophysis tissue are cultured in the same medium but at a distance of about ½ cm from one another Nevertheless, products originating from the hypothalamic tissue evidently reached the anterior hypophysis tissue The latter reacted by producing and/or releasing ACTH which in turn, stimulated the glomerulosa cells to transform into spongiocytes which produced and released ketosteroids into the medium SCHNEIDERMAN then asked whether extracts of hypothalamic tissue can exert similar effects GAILLARD replied that this type of analysis is under way WEISS inquired whether hypothalamic cells establish connections

a number of cases he decapsulated glands of 15 day rats by removing the glomerulosa and a portion of the fasciculata and transplanted them into the anterior chamber of young rats Some of the cortical cells in the transplant survived for as long as 6 months He mentioned that other workers have obtained similar results with fragments of decapsulated glands

TONUTTI suggested that under normal conditions there is no migration from the capsule toward the medulla this probably occurs only if the internal part of the gland is removed He showed a slide of the adrenal cortex of a normal mouse with the three layers present after hypophysectomy there are only a prominent glomerulosa and reticularis If ACTH is added all the cells become of the fasciculata type just as Gaillard found in tissue cultures Tonutti believes that when the adrenal operates under normal conditions and all three layers are present, it is the quantity of ACTH which controls the development of the several layers When there is a strong stimulation, the glomerulosa and reticularis disappear this means that the glomerulosa and reticularis cells have transformed into fasciculata tissue after hypophysectomy there is progressive and retrogressive transformation

Tonutti then called attention to the fact that he has collected data from the literature concerning the time sequence of development of the different endocrine organs in the human embryo (Tonutti and Fetzer 1956) The data are presented as three diagrams Figure 2 shows the formation of the anlage Figure 3 shows the first steps in functional differentiation Figure 4 shows the maturation of the organs after birth In the case of the adrenal, the fetal cortex disappears after birth (involution of inner zone) and the permanent cortex is built up by the outer layer of the gland (development of the outer zone) Full maturation of the cortex takes about 14 years This means that it is completed at the time of puberty This agrees with the fact that the metabolic compounds of adrenosteroids in the urine attain the qualitative and quantitative pattern characteristic of adults at the time of puberty WEISS asked whether the same cortical cells are present during those 14 years TONUTTI stated that the same population of cells probably persists

The experiments of Case (1952) one of Willier s students were then reviewed by WILLIER He noted that development of the adrenals of chick embryos is definitely retarded by the eleventh or twelfth day of incubation in the absence of the pituitary For example the amount of ascorbic acid does not rise but remains at the level characteristic of adrenals of normal 12 day embryos accumulation of ascorbic acid is one of the known indexes of the functional activity of the adrenals

cell type reappears and develops everywhere in the cortical zones This indicates that the glomerulosa type cells can react and develop secondarily into this type of cell

MARTINOVITCH raised a question about the role of the capsule in the development of the cortex He stated that in the past many investigators believed that the capsule could give rise to cortical cells However, the evidence obtained both by in vitro studies and following grafting shows quite clearly that in the rat new cortical cells arise only from pre existing ones (Martinovitch 1955) In 5 to 6 day old explants of whole adrenals the cortical layer undergoes profound degeneration except for a narrow strip of tissue close to the capsule After transplantation of the explants to host animals there occurs a rapid proliferation of these persisting cortical cells, especially those which form the first six to eight rows of cells bordering on the capsule Although some cell division occurs in the capsular layer, the number of dividing cells here is not great There is no clear cut evidence that cells of the capsule are transforming into cortical cells Thus in the regenerating gland the surviving cortex appears to be the sole source of origin of all new cortical cells

WILLIER stated that it was his general impression that there are a few cortical cells left behind next to the capsule WEISS inquired whether they are stem cells WILLIER replied in the affirmative and stated that he believes that they are probably stamped as cortical cells WEISS then inquired whether mitoses are normally found in these cells TONUTTI replied that in the human adrenal there are always some undifferentiated cortical cells within the capsule (Bachmann 1954) MARTINOVITCH stated that such dividing cells are seen by the thousands following transplantation of explants WEISS then asked whether there is a slow continuous mitotic activity here or whether this activity must be activated by some extraneous stimulus TONUTTI replied that some dividing cells are always found there but that more activity occurs when the graft is stimulated WILLIER commented on the fact that there has been a good deal of controversy about the origin of the new cortex but that the part of the cortex adjacent to the capsule has generally been regarded as a proliferative zone whereas the cells at the inner portion of the cortex are used up somehow Thus the cortex is visualized as constantly gaining cells peripherally and constantly losing them centrally

MARTINOVITCH emphasized that under experimental conditions the inner cortical cells are not used up They may survive for long periods of time without undergoing mitotic division (Martinovitch, 1955) In

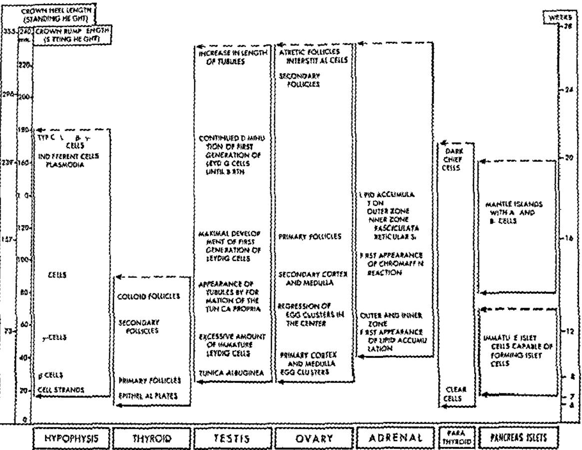


FIG 3 —Diagram showing successive steps of *differentiation* of specific structural (and in part histochemical) constituents of endocrine glands in relation to age and length of human embryos Redrawn from Tonutti and Fetzer (1956) Mantle islands are fetal islets with B cells in the center surrounded by a thick layer of A cells (Ferner 1952)

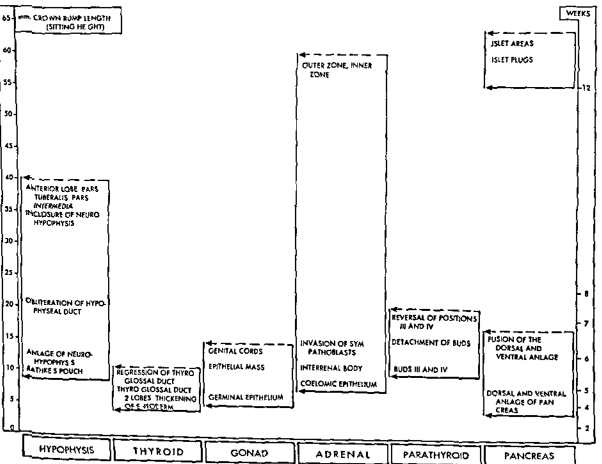


Fig 2 —Diagram showing *formation of the anlage* of endocrine glands in relation to age and length of human embryo Redrawn from Tonutti and Fetze (1956)

WEISS inquired whether this reduction is for the whole gland or on a per cell basis WILLIER informed him that it is for the whole gland WEISS then asked whether the growth of the organ is reduced WILLIER answered that the wet weights of pairs of adrenals are less than normal after the fifteenth day of incubation and fail to increase above the level attained by normal 15 day embryos MOOG pointed out that the amount of ascorbic acid reported by Case is the total for the entire gland medulla as well as cortex as determined by the method of Roe and Kuether (1943) and that his results are expressed as so much ascorbic acid per milligram of adrenal tissue Consequently, the concentration of ascorbic acid in the cortex itself remains unknown WILLIER also mentioned Case's observations on the lipid content of the adrenals fol lowing hypophysectomy The amount of lipid containing cortical ma terial is greatly diminished in partially decapitated embryos at least by the eighteenth day of incubation Whether it is diminished at earlier stages is not known When ACTH is injected subcutaneously on the twelfth fourteenth and sixteenth days of incubation larger amounts of lipid containing cortical material are present by the eighteenth day of incubation but the total amount is not equal to that of normal embryos Despite the apparent increase in cortical elements after treat ment with ACTH there does not appear to be any increase in total size of the glands These observations indicate that the adrenals of pituitaryless embryos can accumulate ascorbic acid and lipids to a lim ited extent but that they cannot accumulate them in the quantities characteristic of normal embryos Thus it appears that the anterior pituitary plays some role in the further development of the adrenals by accelerating the rate of their development and the accumulation of these substances This means of course that anterior pituitary hormones are relea ed into the circulation of the embryo in effective amounts

The discussion then turned to the work of Moog on the effects of ACTH on adrenal growth and adrenal secretion in the intact chick embryo (Moog and Ford unpublished) An ACTH solution is injected into a vein on the chorio allantois at daily intervals from 10 days on the dose is 0 25 mg of ACTH per day The relative weights of the adrenals of treated embryos are consistently greater than those of the controls from 12 days on (Fig 5) At 13 days the *P* value of the mean difference between experimental and controls is above 0 05 but for all other days the differences between experimentals and controls are highly significant Although Case (1952) found that the adrenal weight in his partially decapitated embryos did not begin to fall behind that of the intact controls before 16 days the present results demonstrate

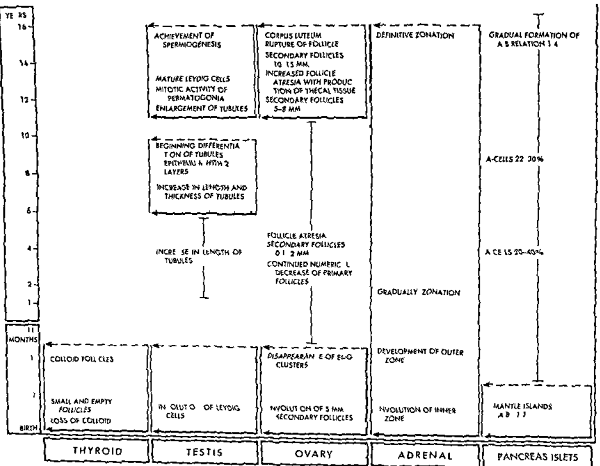


FIG 4.—Changes in endocrine glands after birth and maturation of the structural elements of endocrine glands in relation to age up to puberty Redrawn from Tonutti and Fetzer (1956)

else WEISS expressed his concern about the effect of ACTH injections on body weight MOOG stated that the body is somewhat smaller than normal from 15 days on WEISS then commented that anything that depressed the growth of the rest of the embryo would of course, raise the values for relative weights of the adrenals even if there is no effect on the adrenals MOOG agreed but pointed out that the percentage differences in adrenal weights are considerably greater than those in body weights WEISS then asked whether the absolute weights of the adrenals are slightly elevated or slightly depressed MOOG answered that they are strongly elevated At 15 days for example, the adrenals of injected embryos are twice as heavy as those of controls, and the difference is highly significant She also mentioned that ACTH elevates liver glycogen but stated that the mechanism of its action is not clear It also causes an enlargement of the spleen for reasons which are not understood Both these effects are apparent by 13 days i e at a time when the effect on the absolute weight of the adrenal itself is not clearly significant WILLIER then recalled that Case (1952) had reported that the spleen was greatly enlarged in hypophysectomized chick embryos

MOOG then presented evidence of heightened secretion from adrenals stimulated by ACTH injections The test she uses for secretion from the adrenals involves the increase in phosphatase in the duodenum where of course the enzyme is extremely concentrated Duodenal phosphatase in the mouse increases about a thousand fold between the fifteenth day *in utero* and the nineteenth day after birth Furthermore this increase occurs in two jumps—one just before birth and the other just before the normal time of weaning (Moog 1951) The second saltation which takes place in the nestling has been examined very closely It can be made to occur precociously by administration of cortisone or ACTH and it can be prevented by adrenalectomy at 12 days i e 2–3 days before the rise normally starts (Moog 1953) In the chick embryo there is just a single period of increase culminating about a day after hatching (Moog 1950) This rise can be brought about precociously by administration of hydrocortisone but this increase in phosphatase is not produced directly by the corticoid The hormone actually speeds up the total pattern of differentiation of the intestinal epithelium Phosphatase activity simply goes along as an integrated part of the total pattern of differentiation (Moog and Richardson 1955)

Thus increase in duodenal phosphatase can serve as an indicator of adrenal activity The effect of ACTH on duodenal phosphatase has been investigated The results are summarized in Figure 6 At 15 days

that the adrenals are able to respond to stimulation by exogenous ACTH well before that time Although it is suspected that the stimulation affects only the cortex, no quantitative data are available concerning that point

WEISS inquired about the drop in relative weights of the adrenals in control embryos at 15 days MOOG replied that the matter is not very

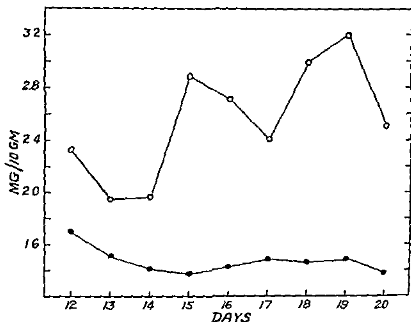


FIG 5—The influence of ACTH on relative adrenal weight in the chick embryo *Open circles* embryos injected intravenously with 0 25 mg ACTH per day beginning at 10 days *solid circles* control embryos injected with acidified saline The ordinate represents milligrams adrenal weight per 10 gm of body weight Absolute values of the adrenals of injected embryos were also higher than those of controls at each stage tested but body weights were lower (Moog unpublished)

clear According to the data of Case (1952) relative adrenal weights do not change much during this period but the data of Sun (1932) and Venzke (1943) likewise show a decrease in relative weights during the early incubation period with a minimum at about 15 days Both Case s data and those of Moog suggest that adrenal growth is controlled by a different set of factors before and after 15 days

WILLIER inquired how cleanly the adrenal complex can be isolated MOOG answered that it is not very difficult to isolate it after 15 days because it is sharply circumscribed at that time But at earlier stages it is difficult to be certain that the whole gland is removed and nothing

1955*a*) Consequently these experiments show that the estrogen effect, which requires an adrenal responsiveness to ACTH can be demonstrated as early as standard stage 25 Morphologically this is a stage when the adrenal is still in an unpaired condition without any intermingling of medullary and cortical components WEISS questioned why by comparative standards an amphibian larva just starting to feed would be any less mature than a 13 to 15 day chick embryo SEGAL answered that this is indicated by the comparative morphogenesis of the glands themselves They are much less developed in the amphibian larva SCHNEIDERMAN then questioned the statement of Segal that onset of adrenal function begins at 13–15 days of incubation in the chick He pointed out that Moog had not said this Instead she had said that the very nature of her test made it impossible to demonstrate adrenal func

TABLE 1

ONSET OF ADRENAL FUNCTION

Animal	Age	Standard Stage	Reported by
Rat	5 days postnatal	35	Gaillard
Chick	13–15 days of incubation	34–35	Moog
Amphibian	Ca 12 days post fertilization	25	Segal

Witschi (1956)

tion prior to 15 days Adrenal function could begin on the eighth or ninth days but as far as her test is concerned onset of function at such an early stage could not be detected

The question was raised by WILLIER whether the adrenals of the treated animals and primarily the adrenal cortex are responsive to administration of ACTH before the anterior pituitary itself is releasing ACTH into the circulation MOOG replied that this is quite possible TONUTTI inquired whether she had seen any increase in the size of adrenal cells in her experiments MOOG replied that the glands had not been examined from that point of view as yet TONUTTI emphasized that it is important to do so because there is a strong relationship between adrenal stimulation with ACTH and the nuclear size of adrenal cells The size of the nucleus is a very specific indicator of the action *of ACTH on adrenal cells much more specific actually* than the weight of the glands MOOG stated that she intends to examine the adrenals of treated embryos much more closely to see whether there is any effect of ACTH on them that can be demonstrated directly Now that

there is already an indication of increased secretion by the adrenal cortex Fifteen days is apparently the earliest stage that results with this test can be expected because no increase in phosphatase content can be induced earlier than this, even with hydrocortisone This means that the duodenum is incapable of response earlier than this Thus it appears that under the influence of exogenous ACTH the adrenal is stimulated to secrete enough corticoid by 15 days of incubation to bring about precocious enzyme production by the duodenum

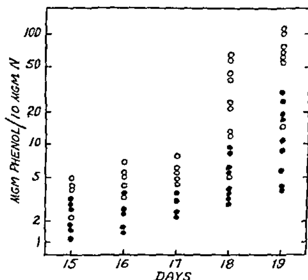


Fig 6 —The influence of ACTH on phosphatase activity in the duodenum of the chick embryo *Open circles* embryos injected with 0 25 mg ACTH per day beginning at 10 days *solid circles* control embryos injected with acidified saline The ordinate represents phosphatase activity as micrograms of phenol liberated from phenylphosphate per 10 μg of duodenal nitrogen per half hour (Moog unpublished)

Segal suggested that the onset of adrenal function in the rat and chick occurs much later than in the amphibian (Table 1) The indicator of onset of function in young amphibian larvae is the reduction of osmic acid by lipid granules in the cortical cells The relationship between ACTH and adrenal function is shown very clearly by a simple experiment involving administration of estrogens to the ambient water of stage 25 frog larvae Starting at this early stage estrogen treatments induce adrenal hyperplasia and hyperfunction (Segal 1953) That this estrogen effect is mediated through the release of ACTH by the pituitary is proved by repeating the same experiment on hypophysectomized larvae In this case no adrenal enlargement occurs (Chang and Witschi

IV

Ontogeny of Selected Endocrine Glands The Gonads

Attention was next turned to the ontogeny of the gonads by Dr Willier He recalled that this topic has received a great deal of atten tion because of the pioneer work of Lillie (1916 1917) on the role of sex hormones in differentiation of sex organs and ducts as demonstrated by his work on the freemartin Burns then discussed evidence for the onset of secretory activity in the embryonic testis He cited three lines of evidence The first may be called presumptive evidence' and is clearly of historical interest It goes back to the time of Bouin and Ancel (1903) who were apparently the first to suggest the hypothesis that since the embryonic testis possesses interstitial cells like those of the adult testis it may well be the source of a hormone controlling sex differentiation This suggestion was based largely on studies of the testes of pig embryos and was proposed thirteen years before Lillie (1916) and Keller and Tandler (1916) presented real evidence for such a theory in their classic studies on the freemartin

It is now known that in a number of mammals cells with the char acteristic appearance of interstitial cells are found in the embryonic testis from virtually the beginning of histologic sex differentiation For example they have been found by Jost (1946) in the rabbit and by Bouin and Ancel (1903) in the pig They are present in the testes of young opossums 10 days after birth at which time the testes are quite embryonic (Burns 1950 Fig 1) The testis cords at this time are still solid and almost unconvoluted retaining the primitive arrangement of simple arches or loops This arrangement leaves a large interior space occupied by large numbers of interstitial cells which are intermingled with the stromal tissue This condition obtains at a stage of develop ment when the external genitalia are morphologically indifferent as is the urinogenital sinus The first prostatic buds do not make their ap pearance until about 7 days after this stage and the paired sex ducts are present in a sexually undifferentiated condition only In fact the

a stage has been found where there is no stimulation of growth, she plans to go back from there to determine whether, by other criteria, she can find signs of an earlier stimulation

Moog also mentioned briefly that, as far as the rat is concerned, an interrelationship between adrenals and the pituitary has been rather clearly shown at about 18 days, i e, prior to birth, by Kitchell and Wells (1952*a* *b*)

Hormone production by the medulla of the adrenal glands was then discussed by WILLIER He pointed out that noradrenalin is produced by the medulla of most fetal mammals, as shown by Shepherd and West (1951) After birth noradrenalin is present in much lower quantities than at earlier stages of development, whereas adrenalin is now present in considerable amounts Adrenalin can be detected in the adrenals of chick embryos by the sixth or seventh day of incubation He raised several questions about the significance of its production so early in development Is it playing any role in development at this time? Does it have an effect on vasoconstriction at an early stage? Does it play any role in the pattern of sugar metabolism in young embryos? Or is there no release of adrenalin from the medulla at these early stages?

Another interesting experiment yields evidence on this point of a different sort When the embryonic testicle of a young rat at the beginning of its sexual differentiation is transplanted between the lobules of the seminal vesicle of an adult castrate male after castration atrophy of the seminal vesicle has occurred, a remarkable thing is seen In a short time there is complete recovery of the epithelium of the seminal vesicle in the immediate vicinity of the grafted testis which appears to be producing a substance not distinguishable from the adult male hormone as far as its effects on the vesicle epithelium are concerned Furthermore histologic examination of the grafted testis reveals that interstitial cells not only are present but are actually considerably hypertrophied under the influence of the host hypophysis (Jost 1948, Jost and Colonge 1949)

These are not the only experiments that could be cited but they are sufficient evidence for two conclusions (1) the embryonic testis is producing an androgenic hormone from a very early stage of its development and (2) it is, in all probability the interstitial cells that produce this substance The results of the castration experiments where organs developing at a distance from the site of the testis are strongly affected by castration would indicate that the hormone is normally being released into the blood stream The external genitalia and other structures far removed from the gonad are among those strongly affected by castration

The effects of embryonic castration on the whole are of the same nature in the chick with one very important exception namely, that it is the *ovary* which is the more active gonad during the period of sexual differentiation in birds (Wolff and Wolff 1951) The effects of castration of male embryos are relatively slight in the case of birds whereas the effects of castration of the female are extremely marked There is no clue as to why the results should be exactly transposed in birds as compared with mammals although it should be noted that the sex chromosome mechanism is also reversed in the two classes (males are XY in composition in mammals but are XX in composition in birds) Perhaps this is in some way the explanation for the fact that in mammals it is the *testis* which exerts the predominant influence in somatic sex differentiation whereas in birds it seems to be the *ovary*

WEISS inquired concerning the situation in fish BURNS commented that the gonads of fish are very elongated and are closely applied to the kidneys hence embryonic castration of fish would be very difficult to perform for technical reasons WEISS insisted that there have been cases of testes removal in various fishes BURNS admitted this but stated that

Mullerian ducts have but recently established contact with the wall of the urinogenital sinus Administration of estradiol propionate from birth onward largely or entirely abolishes this interstitium (Burns, 1956*b*)

The second line of evidence for the interstitial function of the embryonic testis is found in the results of early castration Successful castration has now been achieved in embryos of three species of mammal—the rabbit (Jost, 1947*b*), the mouse (Raynaud and Frilley, 1947), and the rat (Wells, 1950) In general, castration prior to morphologic sex differentiation is followed in the male by an almost complete failure of sex differentiation The male duct system (Wolffian duct derivatives) atrophies and disappears, prostatic glands fail to develop, and the urinogenital sinus is of female type The external genitalia also assume the female form Moreover, in the absence of the embryonic testis the Mullerian duct derivatives persist and are well developed Castrate males thus *eventually come to resemble closely normal females* Evidently, the embryonic testis is the controlling influence in somatic sex differentiation in mammals, and presumably it is the interstitial cells of the testis that are involved In rabbit embryos, castration as early as the twentieth day of gestation has all the effects mentioned above However the effects of castration are progressive depending on the time the operation is carried out The effects are limited to a fairly narrow period when morphologic sex differentiation is most active For example, if rabbit embryos are not castrated until the twenty third day of gestation the results are slight male parts are all present although they may be somewhat undersized Castration on the twenty second day leads to serious deficiencies in the male genital tract, while castration on day 21, or especially on day 20 is followed by almost total suppression of male development (For summary and references consult Willier, 1955)

A few words are in order concerning the point that it is probably the interstitial cells of the embryonic testis which are responsible for its effects on genital development This is best illustrated by certain other experiments of Jost After hypophysectomy of the rabbit embryo by decapitation there is a distinct arrest of development of the male genital tract although the consequences are by no means as severe as after early castration Examination of the testes of hypophysectomized males reveals that there is an important reduction in the number and size of the interstitial cells present The defective development of the genital tract in hypophysectomized males is correlated with failure of the interstitium to develop normally (Jost 1951, 1953)

ing point for those seeking interpretations of the results obtained in a variety of directions

WILLIER showed an illustration of a testis formed by transformation of the freemartin gonad (Willier, 1921) The seminiferous tubules are typical in structure, except that they do not contain germ cells Typical Sertoli cells are present Here and there are cells which he interprets as interstitial cells He contrasted its appearance with that of a normal testis of corresponding age which contains typical seminiferous cords and occasional primordial germ cells

PRICE then discussed her studies on sex differentiation in the rat by the application of organ culture methods An important aspect of this problem—the question of whether fetal rat testes secrete hormone—was resolved by Wells and his collaborators (Wells, 1950 1957, Wells and Fralick 1951), who succeeded in castrating extra uterinized fetuses They found that rat testes secrete a hormone which they concluded stimulates growth of the accessory reproductive glands of the male However they could not castrate fetuses that were younger than 18 days which represents a relatively advanced stage in sex differentiation Price selected the watch glass method of organ culture for her studies on sex differentiation because it has several distinct advantages (1) entire fetal reproductive tracts can be isolated at early stages of development and cultured on clots in which the medium can be controlled, (2) surgical procedures such as gonad removal can be done with a minimum of trauma (3) complicating factors such as the presence of other fetal endocrine glands and maternal and placental hormones are avoided (4) the explants can be seen so clearly on the semisolid clots that development or retrogression of the ducts and the development of the primordia of the seminal vesicles can be observed and photographed from day to day

She explanted approximately 300 male and female fetal tracts, ranging from 13½ to 18½ days in age and cultured them from 1 to 6 days with transfers The tracts which represented all stages from before sex differentiation had occurred to advanced stages of differentiation were cultured under several experimental conditions with both gonads present with only one gonad present with no gonads present with gonads of the opposite sex with no gonads present but with exogenous hormone in the medium etc Her chief purpose was to determine whether fetal gonads are producing hormones which affect the morphogenesis of the tracts and if so to compare those effects with the results obtained by the addition of exogenous hormones The standard medium is cock plasma (the results are no different with capon plasma) and chick em

such operations were performed only on adults and hence were not pertinent to the present discussion

Lillie's classical illustration of the freemartin was then exhibited by WILLIER (Lillie 1917, Fig 4) It shows twin cattle *in utero* They are dizygotic twins, a male and a female, developing simultaneously Their circulatory systems have been injected to demonstrate that a vascular connection exists between the extra embryonic membranes of the two twins Thus there is ample opportunity for exchange of blood between the male and female fetuses and hence for the exchange of any molecules present in the two circulations, including hormones Actually, so far as sex hormones are concerned, there appears to be a one way effect of the male twin on the female twin Interstitial cells appear in the testes of the male before they appear in the ovary of the female Thus there is a precocious formation of the cells in the testis which are concerned with the production of male hormone Consequently, the male hormone is carried into the female partner rather early, and it acts to inhibit female differentiation of the gonad by preventing formation of the ovarian cortex As a rule a testis develops in the genetic female The latter is referred to as the 'freemartin

BURNS pointed out the parallel between the results in the freemartin where the testes of one twin dominate development of the ovaries of the other via their hormones, and the role of the testis in sex differentiation as demonstrated by castration experiments performed on fetal mammals It is evident that the male gonad of mammals is active in an endocrine way during fetal stages, whereas the ovary is not

WILLIER commented on the fact that there is no effect in cattle if two female fetuses develop in the uterus simultaneously, or if two male fetuses develop simultaneously at least as far as sex differentiation is concerned More recently Billingham *et al* (1952 1956) have shown that it is possible to graft skin from one twin onto the other in post natal stages when they are associated with each other *in utero* in such a way that they possess a common circulation This is not possible with ordinary siblings Sharing a common circulation during fetal development enables the twins to tolerate grafts which ordinarily they would not accept Blood cells are also exchanged under these conditions Irwin (1949) and his group have shown that erythrocyte antigens may be identical in the twins which shared a common circulation Thus studies involving freemartins become more and more complex as knowledge increases The pioneer work of Lillie constantly paves the way for investigations of the nature of those mentioned above, and it is the start

substance which diffuses from them in organ culture and causes the maintenance of the Wolffian ducts and the development of the primordia of the seminal vesicles and some of the prostatic buds A further experiment *(A3)* provides interesting evidence concerning the limits of diffusion When the two sides of the tract are widely separated development on the side with the testis continues normally, whereas on the contralateral side the Wolffian duct tends to retrogress and the seminal vesicle is smaller or totally lacking It seems probable that the hormone from the testis is diffusing into the medium and is also passing down the duct system A unilateral effect was also reported by Wells and Fralick (1951) for some unilaterally castrated fetal rats and by Jost (1950) for unilaterally castrated fetal rabbits Jost interpreted his findings as evidence that in vivo the hormone from fetal testes migrates along the genital tract and reaches some parts of the reproductive system in that way as well as by circulatory pathways A fourth type of experiment not illustrated in Figure 7 shows that when testes are absent but testosterone micropellets are present in the medium the Wolffian ducts seminal vesicles and prostates develop normally as in *A1* This is not interpreted as evidence that fetal testes necessarily secrete testosterone The nature of the fetal hormone is not known In these experiments the dependence of the male Wolffian ducts and primordia of the seminal vesicles and prostate on testis secreted hormone or on exogenous androgen seems clear

By contrast the hormone independence of the Mullerian ducts of the female once they are well established is clearly shown in Figure 7 When the tract of a 17½ day female (*B*) is cultured for 4 days development of the posterior ends of the ducts continues and the uterovaginal canal forms *(B1)* Removal of the ovaries *(B2)* has no effect on the Mullerian ducts which appear similar to those in *B1* In both cases the Wolffian ducts retrogress and a few prostatic buds develop (a stock of rats which have spontaneously developing female prostates was used)

This brief and simplified summary suggests that in the fetal rat the testes may normally be secreting hormone which determines the retention of the Wolffian ducts and the development of the primordia of the accessory glands The ovary on the contrary may not be producing a hormone or if it is the hormone is not necessary for the maintenance of the Mullerian ducts However the retrogression of the male Mullerian ducts and of the female Wolffian ducts must be considered These aspects as well as gonad and prostatic development in culture are treated in detail by Pannabecker (1957) and can be discussed here

bryo extract, to which, in some experiments, micropellets of testosterone or estradiol are added Her results leave some important questions unanswered, but at least a part of the problem has been clarified (Price and Pannabecker 1956, Pannabecker, 1957, Price, 1957)

Her results can be summarized by reference to her schematic figure (Fig 7) If a male reproductive tract $17\frac{1}{2}$ days old (*A*) is explanted on standard medium and cultured for 4 days it remains essentially the same size or becomes smaller, but development continues Seminal vesi-

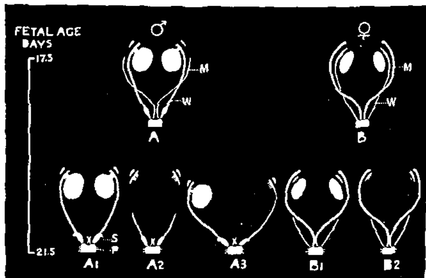


FIG 7 —Diagrammatic representation of reproductive tracts of male and female rat fetuses *A* and *B* male and female tracts at $17\frac{1}{2}$ days the age of explantation *A1 A2 A3 B1 B2* tracts cultured for 4 days *M* Mullerian ducts *W* Wolffian ducts *S* seminal vesicles *P* prostate Redrawn from Price and Pannabecker (1956)

cles form from dilatations on the Wolffian ducts and prostatic buds grow out from the urinogenital sinus Mullerian ducts retrogress anteriorly but the utriculus prostaticus forms posteriorly (*A1*) The results are the same when both testes are removed by carefully severing the efferent ducts and then replacing them This demonstrates that the trauma caused by the removal of the testes does not produce general Wolffian duct retrogression The same results are obtained if only one testis is replaced Thus one testis can insure normal development Complete absence of the testes (*A2*) results in Wolffian duct retrogression and failure of development of the seminal vesicles and some of the prostatic buds These experiments indicate that fetal rat testes secrete a

in the opossum at any rate, it is known that they are reactive under the proper experimental conditions

PRICE then commented that perhaps it is not too surprising that testosterone stimulates male and female Mullerian ducts in her explants because she has been able to observe the Mullerian ducts actually splitting away from the Wolffian duct at the posterior end, suggesting a common origin She pointed out that this agrees with the observations of Gruenwald (1941), who made similar observations in the human and in the chick BURNS stated that this might be expected to occur, especially with a high dose, since different thresholds might be involved and that testosterone in large doses stimulates the Mullerian ducts of the opossum greatly This is an example of the so called paradoxical effect But it does not do so in very low dosages He inquired what happens to the Mullerian ducts in Price's cultures with a very low dose of testosterone He pointed out that in many experiments on the opossum (more recent results of which will be described later on) extremely small doses are far more effective than huge doses which cause all kinds of abnormalities which confuse the picture and even introduce results which are diametrically opposite to the expected results PRICE stated that it was with the hope of obtaining a physiologic level that she explanted fetal testes against female tracts But of course there is no reason for thinking that the threshold of stimulation and the threshold of inhibition are the same in male Wolffian ducts and in female Wolffian ducts in male Mullerian ducts and in female Mullerian ducts

WILLIER then asked whether homologous ducts in genetic males and genetic females actually do give a different response to the same experimental conditions PRICE answered that there are definite differences between male and female Wolffian ducts and male and female Mullerian ducts and that some of these can be attributed to a difference in the male and female genetic constitution BURNS added that this is just the type of result he obtained some years ago in the opossum (Burns 1942) and which has been described in more detail recently (Burns 1956*a*) It holds true for a variety of sex primordia In young opossums of the same litter treated with male or with female hormone under identical experimental conditions as to dosage timing and duration of treatment male primordia under the influence of male hormone always attain a much greater size in male subjects than do the corresponding primordia in female subjects Conversely prospective female structures under the influence of female hormone become larger in female subjects than in males

only briefly The Mullerian ducts of males retrogress anteriorly and develop the prostatic utricle posteriorly under all the culture conditions They are not retained when testes are removed, nor is retrogression hastened by testosterone in fact, some stimulation is found The Mullerian ducts of females are not inhibited by testes placed against them or by testosterone, instead stimulation was apparent in both experimental conditions Female Wolffian ducts undergo slow retrogression in younger explants but retrogress rapidly in older ones They are somewhat stimulated by testes and testosterone, but they are not completely retained under these conditions, and no seminal vesicles form There are possible explanations for some of these findings but more research will be necessary before the questions can be answered However, from these experiments it seems probable that sex differentiation of the ducts and accessory glands in the fetal rat is related to the presence of testis secreted hormone in the male and the absence of such a hormone in the female Jost (1950, 1957) has reported convincing evidence that this is the case in the rabbit

Burns remarked that it was very gratifying to him to see that the rat embryo comes nearer and nearer to being brought into line with the rabbit In spite of the fact that everyone knows that species differences exist and group differences which may sometimes be very great, it is always disturbing when contradictory results are obtained

Price pointed out that the cause of Mullerian duct retrogression in male rats is still a question Jost (1950, 1957) seems convinced that the Mullerian ducts are inhibited by fetal testis hormone in the rabbit He reported a case in which a fetal testis was introduced into the abdominal cavity of a female and caused localized retrogression of the Mullerian duct In her explants by contrast, when testes which are secreting hormone are placed against female Mullerian ducts there is localized stimulation Burns also considers the Mullerian ducts to present a special problem because of the fact that in mammalian embryos it is very difficult or impossible to inhibit them with testosterone or any other known pure androgen By contrast in birds and in amphibians these structures may be completely obliterated by treatment with male hormone This is another striking example of the contradictions that may be encountered in different groups It is a puzzling situation but it may only mean in some cases that no one appreciates all the conditions necessary for development or inhibition of mammalian Mullerian ducts Just a few years ago it was believed that embryonic mammalian gonads also constituted a complete exception in being non reactive to hormones Now,

testis BURNS stated that he would prefer to qualify this remark by adding that the crystalline hormone (testosterone) acts very much like the embryonic testis except in the case of the Mullerian ducts, as mentioned above

SEGAL then recalled that Jost s work on rabbits suggested that mammalian embryos deprived of their gonads, either testes or ovaries would develop the somatic habitus of the female This implies that the syndrome in humans known as ovarian agenesis should include both genetic males and females When Barr and his associates (Moore Graham and Barr 1953) described a sexual dimorphism in the nuclei of epithelial cells of human skin, it became possible to ascertain the genetic sex of humans exhibiting this syndrome When a large group of patients —all raised and living as women—was tested by this means it was found that a large percentage of them were, in fact, genetic males Although these individuals possessed no gonads there was no doubt but that they were sex reversed with respect to their secondary sex characters

Attention was then called by WILLIER to the work of Wolff and Haffen (1952) in relation to the question as to whether or not sex hormones of the embryo and those of the adult are similar or identical They cultivated an embryonic testis in vitro with a bit of embryonic ovarian tissue and found that an ovarian cortex forms on the testis under these conditions Direct contact between the two explanted gonads is not necessary to achieve this result Since the same result can be produced by injections of female sex hormone into male embryos the similarity if not identity of action in the two cases is evident The question of the quantity of hormone produced at these early stages was also raised by WILLIER The amount of hormone produced at first seems to be very low This is indicated by some of his own experiments When little bits of adult testis or adult ovary are transplanted to the chorio allantoic membrane of host embryos the development of the sex of the host is not modified (Willier 1927) Or if a sexually indifferent gonad rudiment is transplanted to the chorio allantoic membrane of a host embryo the sex hormones of the host have no effect on the graft But more recently it has been demonstrated that if a larger quantity of gonadal tissue is grafted to the chorio allantoic membrane hormones produced by the graft can cause sex reversal in the host Such an approach presumably increases the quantity of hormone produced by the graft above the threshold involved in sex reversal

PRICE again recalled that Jost (1953) had reported unilateral effects in unilaterally castrated fetal rabbits and that he had suggested that the

The question was then raised by Segal whether available information is definite enough to state that the secretion of the embryonic gonad is identical with the hormone produced by the adult gonad He seemed convinced that this might be probable and suggested that the one discordant note is an experiment of Jost (1947*a*), in which he found that a deposit of crystalline testosterone does not act like an embryonic testis in maintaining the Wolffian ducts Price stated that she did not know what hormone is secreted by the fetal testes but that testosterone substituted for it, so far as the Wolffian ducts and primordia of accessory glands are concerned She stated that Jost reported that crystalline testosterone maintains the Wolffian ducts but does not cause retrogression of the Mullerian ducts Burns added that, as far as the prostate and other structures in opossums are concerned, crystalline hormones substitute completely for the absence of the testes but do not cause the inhibition of ducts, which seems to be in a separate category Burns does not believe that anyone would hold out for identity in any literal sense of the word Yet that is the only variable in the physiologic effect

An interesting question was then raised by Weiss He inquired whether testosterone is now considered to be exactly identical with the secretion of the adult testis Segal stated that it has been isolated from testis extracts and has also been recovered from blood taken from the testicular vein But Burns answered that it is not so considered to his knowledge He pointed out that there are many effects of the adult hormone even beyond the confines of the genital system There is certainly marked physiologic similarity, but hardly identity Weiss then stated that if this is so the inability to reproduce an embryonic effect by a crystal of testosterone does not necessarily indicate that the embryonic and adult hormones are different The use of testosterone crystals simply cannot prove or disprove the identity or non identity of embryonic and adult hormones

Willier suggested that the preponderance of the evidence indicates that they are similar, if not identical whatever their nature may be He mentioned that Witschi (1950) had argued that the effect of a bit of fetal testis on the epithelium of a seminal vesicle to which it had been added (Jost 1948) is an inductor effect rather than a hormonal effect But, since the same kind of effect can be produced by administration of sex hormones to the castrated adult rat the response of the seminal vesicle to the transplanted fetal testis is the same as to a synthetic hormone

Segal concluded that the over all evaluation of Jost s work leads to the belief that a crystalline hormone does not act like the embryonic

It is believed that this regulation is due in part to a restricted output of thyrotrophic hormone, for which host and implant thyroids compete

WEISS then inquired when pituitary control over gonad activity began BURNS replied that Jost (1953) has evidence in rabbit embryos that the pituitary is quite active during the period when the secretory activity of the testis and the process of sex differentiation are most marked KOLLROS called attention to the work of Fugo (1940), which shows a reduction of intertubular tissue in the testis of pituitaryless chick embryos as early as the thirteenth day of incubation WILLIER stated that he had been toying with the idea that the anterior pituitary may be releasing its gonadotrophic hormones earlier than hitherto suspected in very small quantities which somehow or other are utilized by the developing gonads Willier also stated that Fugo's results should be more thoroughly investigated and controlled by administering gonadotrophins to pituitaryless embryos to determine whether the condition of the gonads could be restored to normal Preliminary attempts in this direction have been made by Vogel (1957) KOLLROS stated that if the absence of the pituitary causes a change in the normal pattern of gonad differentiation it is implied that the gonad is already sensitive to pituitary hormones by that time He inquired whether there is any evidence that it is sensitive earlier According to WILLIER no information is available on this point

WEISS commented that his interest in the time of onset of pituitary control over gonad activity was simply to determine whether there is a sufficient safety margin there i e whether the pituitary is already active by the time the gonad takes up its function as the next station down the line Returning to the question of the quantity of hormone produced early in the development of endocrine organs he pointed out that the statement that less hormone is produced earlier and more later does not necessarily mean that the individual cell is less effective from the start It may only mean that there are fewer cells producing the hormone initially The individual cell that is releasing gonadotrophins may be fully efficient right from the start more hormone would be released as more cells become capable of secretion But perhaps the individual cell does increase its output with time WILLIER suggested that although the total number of cells does increase with time to some extent the increase is probably not sufficient to account for increased hormone production with time According to KOLLROS the work of Etkin (1955) and others shows clearly that the thyroid gland grows remarkably in the stages preceding metamorphic climax in amphibians and

hormone level was higher on the testis side because of migration of the hormone along the genital tract She again recalled that diffusion of hormone from the fetal testis was clearly evident in her explants WILLIER agreed that there is more and more evidence accumulating all the time that, when an endocrine gland is placed close to its receptor, the former affects the latter by diffusion of its hormone through the intervening tissues At a stage in development when the receptor will not respond if located at a distance from the endocrine gland it will respond if placed close to the latter This can only mean that low concentrations of hormone are released at first The concentration is too low to have an effect via the circulatory system but is high enough to have an effect by diffusion to nearby regions WEISS pointed out that this is simply another way of saying that the receptor organ responds above a certain threshold regardless of the route by which the threshold is reached, whether through the circulation or through the tissues spaces He also stated that the experiments in which unilateral effects were obtained in unilaterally castrated fetal rabbits also support this conclusion WILLIER then commented on the fact that this concept of diffusion of hormones from their point of origin has appeared in the literature only quite recently It was first established by Etkin (1939), who transplanted a thyroid gland near the anterior pituitary and vice versa and obtained precocious stimulation of the thyroid Development of this concept has been reviewed (Willier, 1955) It has certain connotations for general thinking about various problems concerned with the release and action of hormones More recently Dossel (1958) has examined this situation in chick embryos He isolates the thyroid vesicle in early stages (from 4 to 4½ day donors) and transplants it together with some mesenchyme to the head or to the coelom of an intact host embryo The thyroid tissue that develops from the implanted vesicle 7 or 8 days later forms a single spherical mass rather than two approximately equal masses, as in the normal embryo It was thought that the host would then have a complement of thyroid tissue equivalent in mass to that possessed by two normal embryos of similar age Instead the combination of the mass of thyroid tissue of the implant and of the normally situated thyroids of the host is only slightly but not significantly more than the total mass of tissue of the normal embryo of similar age This regulation always involves a decrease in the mass of the host's own thyroid whether the original implant is placed in the head or in the coelom In 11 day hosts the aggregate is only about 107 9 per cent of normal in 12-days hosts 103 6 per cent and in 13 day hosts about 104 per cent

to 3 mg/24 hours These are the data of Schmidt and Tonutti (1956) GAILLARD then asked what happened to the development of the boy after removal of the tumor TONUTTI replied that the patient lost his pubic hair but continued to gain in height The testis seemed to become somewhat larger A fairly great number of normal Leydig cells are present in both testes It appears that this boy is changing from a precocious pseudo puberty into a true, but still incomplete, precocious puberty The observations of Wilkins and Cara (1954) are also pertinent, they involve cases of the adrenogenital syndrome It appears that androgens produced by a pathologic tissue mature the organism as reflected by bone age and that if a certain degree of somatic maturation is reached, the hypophysis begins production of gonadotrophins

PRICE asked how long before birth the prostate gland of the human male becomes hypertrophied She thought information about this might suggest great activity of the fetal testis TONUTTI had no information about this MOOG inquired whether such a change in the prostate gland might possibly be due to androgens from the fetal adrenal cortex rather than from the testis She stated that the fetal cortex does very definitely produce androgen according to the work of Bernirschke and Bloch and their co workers (Bernirschke *et al* 1956 Bloch *et al* 1956) Since there is so much of this tissue there might well be a strong androgenic stimulation from this source Consequently she suggested that it would be difficult to draw any conclusions about the activity of the fetal testis from observations on the development of the prostate PRICE then stated that she was simply intere ted in learning whether there is any relation between the Leydig cells of the fetal testis and hypertrophy of the prostate TONUTTI informed her that the peak of development of the Leydig cells is much earlier than the development of the prostate It corresponds with the differentiation of the cells in the hypophysis i e it occurs at about the 10 cm stage WILLIER then suggested that Moog s method of studying the time at which the adrenal cortex can respond to stimulation by ACTH is the approach indicated here for study of the problem of time of onset of gonadotrophic stimulation MOOG inquired whether chorionic gonadotrophin would not complicate the picture to which WILLIER replied in the affirmative provided that they get through the placenta

that, during the climax phases, cytologic evidence points to an increased secretory activity of the gland

SEGAL then mentioned that there is an indication that the fetal gonad is at least responsive to gonadotrophic stimulation in the human The interstitium is obviously stimulated at the time of birth, most likely as a result of its exposure to chorionic gonadotrophins *in utero* Shortly thereafter there is a regression of the interstitium, again indicating that the initial stimulation was the result of gonadotrophic stimulation He pointed out in relation to this comment that it is curious that no observation has been reported about the condition of the testes in newborn anencephalic monsters

TONUTTI discussed this matter in more detail He stated that the peak of development of the interstitial cells in the human testis occurs at a length of 10 cm Regression begins before birth He questioned whether it is actually the chorionic gonadotrophin which stimulates these cells SEGAL commented that this parallels the level of gonadotrophins and TONUTTI agreed that this is so at the 10 cm stage But he pointed out that regression of the Leydig cells in the fetal testis is not uniform Even at birth there is a considerable amount of well developed Leydig cells present In cases of hypogonadotrophic hypogonadism the entire population of Leydig cells shows about the same stage of regression He called attention to the curious fact that in spite of the presence of well developed Leydig cells the latter are not influencing tubule formation in any obvious way Ordinarily when Leydig cells are stimulated by gonadotrophins after birth they induce tubule formation Thus the question arises whether the fetal Leydig cells are producing the same hormones as mature Leydig cells of the adult Recently he observed a five and a half year old boy with Leydig cell tumor and pseudo precocious puberty Near the tumor the tubules had a diameter of about 130 μ and showed advanced, but not complete spermatogenesis At a distance from the tumor the diameter of the tubules was only 70 μ and the epithelium was immature The diameter of the tubules in the other testis without tumor was about 60 μ The tumor appears to produce testosterone this may be concluded from a high peak of androsterone in the chromatogram of the 17 ketosteroids BURNS suggested that perhaps there is a question concerning the time at which the tubules become sensitive TONUTTI answered that they are always sensitive after birth WILLIER inquired about the appearance of secondary sex characters in this boy TONUTTI answered that they are very well developed This boy has a bone age of about twelve years 17 Ketosteroids before operation amounted to 8–10 mg/24 hours After semicastration they fell

tologic sections the osteoclasts are found in close contact with spicules of bone and brush borders are evident From these experiments it appears quite certain that, from the eighth day on, the parathyroid tissue is capable of releasing some agent which causes the bone matrix to change its chemical composition, this is then followed by intensive osteoclastic resorption The same kind of effect is found after combined cultivation of parathyroid tissue taken from human newborns as well as after the addition of commercial parathormone from Eli Lilly and Company (Gaillard, 1955*a* *b* 1957) GAILLARD stated that it was Barnicot (1948) who first described the same type of results in vivo and that Chang (1951), working in Bloom's laboratory, later extended Barnicot's work However he emphasized that the possibility exists in their work that antigen antibody reactions might complicate the interpretation of the results whereas in the absence of these reactions in vitro misinterpretations are much less likely

WILLIER recalled that he had presented some arguments (Willier 1955) in favor of early secretion of parathormone by the mammalian fetus Chief among these is the fact that the parathyroid glands assume adult histologic characteristics rather early in development, actually very shortly after they are detached from the pharyngeal pouches GAILLARD mentioned that it is difficult to get evidence of secretory activity from the histologic picture encountered in avian parathyroid tissue Morphologic evidence of a secretory cycle is much less pronounced in this gland than in the anterior pituitary for example

WEISS then inquired whether the parathyroids of embryonic stages act through the same mechanism as in later stages GAILLARD replied that he could say only that approximately the same kind of effects occur in hyperparathyroidism although they are not encountered simultaneously in the entire skeleton At least in the beginning of the disease there are certain sites of preference where bone resorption obviously occurs more readily WEISS then inquired whether the hormone acts through its effects on some of the cells of the bone or whether it acts directly on the ground substance GAILLARD answered that it is his impression that its action is directly on the bone matrix however he could not be certain that the cells around the matrix were not involved in some way It appears quite certain that the immediate effect is not on osteoclastic activity The initial changes in the bone occur without any obvious activity of osteoclasts WEISS then asked whether its effect might be on the collagen GAILLARD answered that if collagen is interpreted as part of the whole system of structural relations between collagenous fibers and the adjacent mucoproteins it is probably the muco

V

Ontogeny of Selected Endocrine Glands The Parathyroids

The development and secretory activity of the parathyroids was next discussed by Dr Gaillard He attempted to get some information about the secretory activity of the parathyroid glands during development of the chick embryo with the aid of organ culture methods First, he had to develop a method for dissection of the parathyroid glands They cannot be isolated by a frontal approach but can be obtained by a dorsal approach It is then rather easy to excise the glands even from an 8 day embryo In order to check the secretory activity of the parathyroid tissue the gland is placed *in toto* on top of a square fragment of parietal bone excised from the center of the actively growing bone of a mouse embryo near term As a rule the combined culture of active parathyroid and bone tissue leads, within 3–6 days after explantation to a definite lacunar bone resorption This never occurs in control explants of bone tissue without parathyroid tissue As another type of control experiment, parietal bone is combined with explants of thyroid gland tissue In this case bone resorption does not occur on the contrary, there is an enormous amount of ectopic bone formed inside the inner periosteum (Gaillard, 1955*a* *b*) Summarizing the results of a great number of experiments it can be said that the parathyroid gland tissue of chick embryos is active in this way from the eighth day of incubation on When the influence of the gland on the bone tissue is examined in more detail, it is found that the first change is a relatively intense staining reaction of the bone matrix with the periodic Schiff reaction after only 2 days of culturing At the same time the number of cells in the inner periosteum increases considerably and they begin to encapsulate the parathyroid explant which is placed in close contact with the inner periosteum Moreover cinemicrographic records of a number of explants reveal an increased mobility of the intertrabecular cells As a rule some resorption of bone matrix is found even when typical osteoclasts are not visible After 5–7 days of cultivation a very intensive bone resorption occurs and many giant multinuclear osteoclasts are then present In his

VI

Endocrine Organs of Arthropods, with Special Emphasis on Insects

The nature of the endocrine organs of insects and other arthropods was reviewed by SCHNEIDERMAN to provide background information for any comments to be made by him in the future There are three principal endocrine organs in insects that are known at present the brain the prothoracic glands and the corpus allatum The brain contains several groups of neurosecretory cells which produce an endocrine substance which may be called the "brain hormone" In some insects these neurosecretory cells send their axons to an organ just behind the brain the corpus cardiacum where they release neurosecretory material The corpus cardiacum (or corpora cardiaca for they are commonly paired organs) serves as a storage place for neurosecretory substance in the same way as the sinus gland of crustaceans or the posterior pituitary of vertebrates Presumably it releases its storage products into the blood, possibly in modified form The corpus cardiacum is of nervous origin as is the neurohypophysis of vertebrates

The corpus allatum is found in close association with the corpus cardiacum and like the latter is commonly paired It is of ectodermal origin and produces a hormone which may be called the 'corpus allatum hormone or better the 'juvenile hormone for reasons which will be clear shortly

The prothoracic gland a diffuse glandular organ made up of conspicuous large cells and commonly located in the prothorax is an important and very ancient endocrine structure It produces a hormone which will be called the prothoracic gland hormone, hereafter referred to as PGH

The way in which these endocrine glands work can best be understood by considering what happens to an epidermal cell in an insect when it is exposed to these hormones PGH is the growth and molting hormone of insects In the presence of this hormone the insect will grow and molt In its absence growth and molting cease This appears to be true in all insects thus far studied In response to stimulation by

protein part of the system which is attacked primarily TONUTTI then mentioned that Engfeldt and Zetterstrom (1954) believe that parathormone acts primarily on bone cells They reported that microchemical reactions in the bone are first detectable 18 hours after a single injection of parathormone into the intact animal, whereas cellular reactions are recognizable only 6 hours after injection GAILLARD stated emphatically that this is certainly not the case in explants Here the first reaction occurs in the bone and the osteoclasts react only secondarily Osteoblasts are not influenced at all because, in the same explant, osteoblastic formation of bone occurs a short distance from the explant of parathyroid tissue at the same time that resorption occurs near the latter Only fibrocyte like cells are present in the vicinity of the parathyroid explant no osteoblasts are present there Active osteoclasts are seen from the fifth or sixth day on

WEISS then inquired whether osteoclasts and osteoblasts are the same cells in different stages of operation as McLean and Bloom assumed (McLean, 1943) GAILLARD stated that they assume a fusion of osteoblasts leading to formation of osteoclasts He also stated that his moving picture provides no information about the origin of osteoclasts Within a few hours they are present as very actively moving cells They move from one place to another, settle down on a piece of bone matrix do their job, and move again to another fragment of bone matrix The culture media always become exceedingly acid during cultivation of parathyroids. When parathormone was used on cultures the most active concentrations were found to be between 1 5 and 0 02 IU/cc There is some evidence that this hormone is a protein, but there is no information as yet about its structure Eli Lilly and Company is trying to prepare a crystalline parathormone

large amounts of juvenile hormone, and, consequently, every time the larva molts, it molts into a larva But if the corpora allata are extirpated from the larva at any stage, it promptly molts into the next stage i e, into a pupa This pupal molt presumably reflects the response of the insect to PGH and to small amounts of juvenile hormone remaining even after the allata are removed (Fukuda, 1944) By carefully timed surgical maneuvers it is possible to get a larva to molt in what is ap parently the virtual absence of juvenile hormone Thus C M Williams and W H Telfer (unpublished) extirpated the corpora allata from a mature *Cecropia* silkworm larva about 2 weeks before pupation was scheduled to occur Under these conditions endogenous juvenile hor mone soon disappears from the blood of the larva, for, when it molts, it forms a bizarre intermediate between pupa and adult Apparently, in the absence of the juvenile hormone, some of the epidermal cells go directly from making larval cuticle to making adult cuticle without the intervention of the pupal stage

It is of some further interest that a larva can be caused to remain a larva long after it should have pupated, by supplying it with juvenile hormone Whenever most of this hormone is removed the larva then becomes a pupa Thus in the normal life of the insect the corpora allata cease secreting at the end of larval life and the pupal molt ensues When the next molt occurs the corpora allata are still inactive, the endogenous juvenile hormone has all disappeared and the pupa now molts into an adult Thus the larval pupal, and adult molts are as sociated with progressively smaller amounts of juvenile hormone

A question arises as to whether pupal or adult epidermis can be made to secrete larval cuticle The answer probably is Yes Thus Piepho (1939*a* *b*) showed that when fragments of pupal integument are im planted into young larvae in which the juvenile hormone titer is high a larval cuticle is secreted Thus the epidermal cells have not reached a morphogenetic dead end when they become adult they are still capable of producing a juvenile cuticle when exposed to suitable ex trinsic stimuli

So much for the sources and effects of these hormones Their chemical nature can now be considered In 1954 after ten years of effort, Peter Karlson and Adolph Butenandt of the Max Planck Institute at Tu bingen succeeded in isolating from 500 kg of commercial silkworms (about 330 000) 25 mg of a crystalline substance (Butenandt and Karlson 1954) This substance when injected in one to ten parts per million causes insects to molt Indeed if it is injected into the isolated pupal abdomen of a *Cecropia* silkworm which contains no endocrine

PGH, an epidermal cell secretes a new cuticle and molts The kind of cuticle that the epidermal cell secretes indicates the stage of differentia tion of that cell The cuticle of the epidermal cell is thus a fingerprint of the cell s morphogenetic potentialities The kind of cuticle that the epidermal cell produces depends on whether or not corpus allatum hormone is present In the case of silkworm larvae for example if the epidermal cell is exposed to PGH *only*, the insect as a whole will pro gress in the adult direction and the epidermal cells will produce a cuticle with adult characteristics If, however there is present, in addi tion to PGH a large amount of corpus allatum hormone this same epidermal cell will produce a larval cuticle Finally, if PGH is present together with a small amount of juvenile hormone, the very same epidermal cell will make a pupal cuticle If the differences between the scaly cuticle of a moth the hard tanned cuticle of a pupa and the smooth cuticle of a caterpillar are recalled it will immediately be rec ognized that in these different hormonal milieus the epidermal cell is making very different kinds of cuticle Thus the basic stimulus for growth and differentiation of epidermal cells (and the rest of the insect as well) is PGH but the direction of development—whether larval pupal, or adult—is determined by another hormone, the juvenile hor mone of the corpus allatum If there is a great deal of juvenile hormone present a larval cuticle is produced if a small amount is present, a pupal cuticle and if none at all is present an adult cuticle

The brain hormone is a trophic hormone and has as its specific target the prothoracic glands The brain hormone stimulates the latter to secrete PGH presumably in a way not unlike the stimulation of the adrenal cortex by ACTH

Postembryonic growth and molting of insects can be understood quite simply in terms of these three hormones Indeed there is evidence that even during embryonic life these hormones play a role For example some grasshoppers molt about halfway through embryonic life in re sponse to secretion of PGH by the embryonic prothoracic glands

One of the most striking features of insect development is that growth and molting in insects are cyclical Periods of active growth and molting alternate with periods of quiescence The cyclical molting results from the cyclical activity of the neurosecretory cells in the brain Periodically these cells are active and periodically they stimulate the prothoracic glands to secrete PGH What the cells of the insect do in response to this stimulation depends on the amount of juvenile hormone that is released by the corpora allata Consider for a moment the commercial silkworm *Bombyx mori* During larval life the corpora allata release

ly secretes ecdysone There are also numerous neurosecretory cells in the brain and associated structures of Crustacea and these appear to regulate the activity of the Y organ, perhaps in a fashion similar to the regulation of the activity of the prothoracic gland of insects by the neurosecretory cells of the brain Growth and molting in the Crustacea are thus under the control of neurosecretory cells and a homologue of the prothoracic glands

WEISS inquired whether the crustacean glands have actually been tested experimentally for their endocrine functions This question was raised because Schneiderman had referred to them as "homologues of insect organs SCHNEIDERMAN answered that Gabe (1952*a* *b* *c* 1953) in Paris the discoverer of the Y organ has shown that this organ resembles the prothoracic glands histologically and his co-worker Échalier (1954 1955) has conducted extirpation and implantation experiments which demonstrate that the Y organ controls molting at least in the decapod Crustacea (see review of Knowles and Carlisle, 1956) Moreover Karlson (1956) has extracted from the shrimp *Crangon vulgaris* a hormone which causes insects to molt, presumably ecdysone from the Y organ

SCHNEIDERMAN also mentioned that there are certainly other hor mones in insects that have received less study For example the ovaries of many insects appear to produce a hormone that acts on the corpora allata, if an insect is castrated there is a hypertrophy of the corpora allata He also amplified further the analogy between the corpus car diacum–corpus allatum complex of insects and the neurohypophysis adenohypophysis complex of the vertebrates (Wigglesworth, 1934, Han trom 1941) In the insect there is a portion of nervous origin—the corpus cardiacum—closely associated with a portion of ectodermal origin—the corpus allatum In the vertebrates there is a nervous rudi ment—the neurohypophysis—that fuses during development with an ectodermal rudiment—the adenohypophysis Moreover in both the vertebrates and the insects neurosecretory cells from the brain send their axons to the nervous portion

WILLIER inquired what kind of cells are found in the corpus car diacum SCHNEIDERMAN answered that there are neurosecretory cells there the so-called chromophil cell In addition there are also axon terminations of neurosecretory cells who e cell bodies lie in the brain There are al o some chromophobic cells which may play a role in secre tion The chromophobes appear to be of neuroglial origin and are thus not neurons But the entire gland is of nervous origin The corpus allatum is entirely different in appearance It is an ectodermal structure

organs, it causes the molt of this structure into an adult abdomen (Williams, 1954) This crystalline material is unquestionably PGH, and Karlson has given it the name "ecdysone" (Karlson, 1956) Fortunately, it is a small molecule and has no nitrogen Its elementary formula is $C_{18}H_{30}O_4$ and it appears to be an alpha beta unsaturated ketone with hydroxyl and methyl groups It is active endocrinologically in virtually all insects that have been studied Moreover, Karlson has recently isolated ecdysone from crustaceans, in which it apparently *controls molting just as it does in insects (Karlson, 1956) Thus it* seems fair to say that in an evolutionary sense the prothoracic gland hormone ecdysone is probably the oldest hormone that is known, for the insects and the Crustacea must have diverged a long time ago Indeed, ecdysone probably triggered off molting in the trilobites! Aside from its ancient origin, ecdysone is a significant hormone molecule for another reason it is responsible for more animal growth in our particular part of the universe than any other molecule

The juvenile hormone is a molecule of no less interest It is a conservative factor which permits growth and molting but prevents differentiation It represents at least for the insect the fountain of youth As long as the insect maintains production of the juvenile hormone, maturity will be delayed Very recently Carroll Williams (1956) obtained an active extract of juvenile hormone by the simple maneuver of extracting abdomens of male *Cecropia* moths with ether Why the juvenile hormone should be stored in the abdomen of male moths is not yet known About 0 2 cc of clear, yellow oil can be obtained from each abdomen and this oil has potent juvenile hormone activity For example injection of about 0 1 cc into a silkworm pupa that is about to molt into a moth causes the pupa to molt into another pupa The extract works not only on silkworms but on all other insects studied The juvenile hormone like ecdysone, appears to be a small molecule that is not species specific

Nothing is known about the chemical nature of the brain hormone and all efforts to obtain an active extract of this substance from brains have failed completely All that is known about the brain hormone is that neurosecretory material from the brain (which may or may not be brain hormone) moves down neurosecretory axons to the corpora cardiaca where *it appears to be stored* Berta Scharrer (1952) has shown that if the connections between the brain and the corpus cardiacum are cut neurosecretory material accumulates above the point of section

In the Crustacea molting is under the control of an organ called the "Y-organ which is homologous to the prothoracic glands and apparent

active SCHNEIDERMAN replied that Jones (1956) has done this He conducted some revealing experiments on the embryonic development of grasshoppers He showed that after the first third of embryonic life, after the embryo has acquired well formed legs and eyes the only endocrine organs that are evident are some large neurons in the brain destined to become neurosecretory cells But soon after this stage when the embryo rotates in the egg (the process called 'katatrepsis) and when the most dynamic aspects of morphogenesis have been com pleted the corpus allatum and the prothoracic glands appear almost simultaneously Jones showed that if an embryo is ligated between the thorax and the abdomen just after katatrepsis both halves continue their development perfectly normally The only difference is that the isolated abdomen fails to undergo an embryonic molt Cell division goes on however and the various embryonic structures in both halves of the ligated animal assume their definitive appearance These experi ments led to the conclusion that a good deal of embryonic morphogenesis of insects can take place without the intercession of any of the known endocrine organs In fact Donald Bucklin (unpublished) showed several years ago that an isolated limb of an embryonic grass hopper will continue cell division and differentiation in a hanging drop of saline in the complete absence of any endocrine structures It ap pears therefore that the embryonic development of insects does not demand the activity of endocrine organs unless these structures are very diffuse throughout the body of the insect during embryonic life No distinct and definitive endocrine organ is implicated in the em bryonic development of insects The role of diffuse endocrine structures remains to be seen

WEISS inquired about the old claim of Frew (1928) who reported that the evagination of imaginal discs does not take place in larval blood but does take place in pupal blood SCHNEIDERMAN pointed out that this of course involves postembryonic development He stated that no one has ever been able to repeat Frew s experiments although several have tried It must have been a lucky happenstance that cells developed under the conditions of his experiments WEISS then asked whether anyone has been able to get a result in tissue culture com parable to those of Gaillard in which an isolated part will react to hormones SCHNEIDERMAN answered that Loeb and Schneiderman (1956) maintained insect tissues alive for as long as 2½ months in tissue culture but that nothing happened The cells merely sit there occasionally sending out protoplasmic processes WEISS then asked whether these cells failed even to produce a cuticle SCHNEIDERMAN

which arises by budding of cells between the mandibular and maxillary segments These buds then separate from the epidermis The prothoracic gland consists of an anastomosing network of very large cells which is richly supplied with tracheae There are cell boundaries, but sometimes it appears almost syncytial The cells are quite characteristic and often contain lobed nuclei of irregular shape WILLIER then asked whether there is any evidence of a secretory cycle SCHNEIDERMAN replied that the cells of the prothoracic gland have a secretory cycle as do the neurosecretory cells Where these cycles have been studied in detail, they closely parallel the morphologic events of molting WILLIER also inquired whether the cells of the corpus allatum contain secretory granules SCHNEIDERMAN did not know

WILLIER *then asked about the time order in which the endocrine* glands of insects develop SCHNEIDERMAN replied that this has been studied embryologically in some detail by the Japanese, who have been very interested in everything to do with the life of silkworms, and more recently by Jones (1956) in the grasshopper The glands are all formed during embryonic life In the case of the grasshopper, there is some information about the functional activity of these cells in embryonic life WILLIER asked Schneiderman what he meant by ' embryonic life ' He stated that he means the time from fertilization until the little grasshopper hatches from the egg i e the first third of life, when the legs and eyes have formed but prior to katatrepsis, when the embryo rotates in the egg The only endocrine structures that are evident are the neurosecretory cells In the development of an insect like a grass hopper as long as the animal is inclosed within the egg and is still in the process of consuming its yolk it can be called an 'embryo In the *Cecropia* silkworm as long as the insect is within the egg and has not emerged *it can be called an embryo* WILLIER *then asked whether* the animal emerges from the egg as a caterpillar SCHNEIDERMAN answered in the affirmative and stated that a caterpillar is certainly no embryo It is a highly developed free living organism When the caterpillar has emerged from its egg all the endocrine organs are fully formed There does not appear to be an obvious postembryonic development of any of the endocrine glands of insects They are then in their definitive condition

KOLLROS asked whether the cells of these glands have also attained definitive size at this time SCHNEIDERMAN stated that the cells will increase in size and in some cases in number as the animal increases in mass WEISS asked whether *anyone has gone back into the egg and* fractionated development so to speak to see when these glands become

VII

Ontogeny of Selected Endocrine Receptors The Gonads and Gonoducts

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The subject of the gonads and gonoducts was introduced by Dr Burns He stated that the effects of sex hormones on the development of the genital system have been examined in at least six species of placental mammals as well as in the opossum during the last 15 years These hormones easily produce profound transformations of the sex of the genital tract but historically have had almost no effect upon the development of the gonad This is distinctly contrary to the situation in the lower vertebrates (fishes amphibians and birds) in which steroid sex hormones transform the gonad quite easily For example if a genetic male opossum is treated with estradiol dipropionate from the age of 4 or 5 days after birth until the age of 30 days the testes are unmodified except that the interstitial tissue is not quite normal and patches of germinal epithelium persist here and there on the surface of the gonad Normally the germinal epithelium is completely lacking by this time Moore obtained like results with opossums and similar observations were made on treated rats by Jost and on treated mice by Raynaud (see Burns 1950 for references and discussion) All investigators involved in this early work agreed that a testis modified to such a slight extent is essentially unmodified

If treatment is initiated somewhat earlier after only 2 or 3 days of postnatal life there is more modification than before but the gonad is still fundamentally a testis The growth and convolution of the testicular tubules are then greatly repressed and inhibited They remain arch shaped tubules confined to the periphery of the gonad Again there is a persistence of a slightly thickened germinal epithelium on the surface of the testis

When treatment with female sex hormone is begun almost immediately after birth the development of the tubules is greatly repressed and the amount of interstitium is much reduced The tunic layer—a thick fibrous layer—is very thick and swollen The germinal epithelium persists over the entire surface of the testis (see Burns 1956b Figs 11

stated that if they are in the midst of doing this at the time they are placed in the culture medium, they stop doing it They are not dead, because if they are transplanted back into an insect, they develop He feels that there are good reasons for this behavior None of the culture media used have duplicated the proper hormonal environment The insect's blood, strangely enough, is a highly toxic medium in which to grow insect tissues He feels, furthermore, that it will be difficult to get any kind of true insect tissue culture until ecdysone is available This hormone will probably prove necessary for the culture of most insect tissues WEISS then wanted to know what evidence proves that this is a growth hormone that will act outside the body and actually make things grow that would not do so in its absence SCHNEIDERMAN called attention to the classical experiments begun by Goldschmidt (1915), who showed that spermatocytes of the *Cecropia* silkworm isolated in hanging drops sometimes developed into sperm and some times failed to do so for reasons that were not understood at the time It was subsequently shown by Schmidt and Williams (1953) that when ever the pupal insect has initiated its adult development and has quantities of PGH in its blood spermatocytes develop into sperm when isolated in the blood But in the blood of a pupal insect which has not *begun its adult development spermatocytes fail to differentiate* In this instance of course it is not a matter of growth but almost solely of differentiation under the influence of the hormone

WEISS insisted that this was exactly his point and asked how Schnei derman could be certain that once Karlson does get more ecdysone and it is added to the culture medium growth will actually be stimu lated SCHNEIDERMAN could only say that it was their hope that the addition of this hormone would enable the cultures to grow He pointed out that actually the hormone is no longer called a growth hormone It is called ecdysone derived from the word *ecdysis* which means 'molt Ecdysone then is the molting hormone Nevertheless there is reasonable evidence which has been summarized by Wigglesworth (1954) that this hormone is in fact a growth hormone

anyone that the gonads had ever been genetic testes were it not for the fact that there is one infallible way to demonstrate the original sex of the opossum embryo Among all the parts of the urinogenital system the only structures which seem to be completely unreactive to sex hormones during this entire early period of development are the scrotal sacs in the male and the pouch structures in the female (Moore 1941 Burns 1942) Thus a treated individual with ovaries but possessing scrotal sacs is presumably a genetic male whereas a treated individual with ovaries but possessing pouch structures is most certainly a genetic female (see Burns 1956*b* Fig 21) In both genetic males and genetic females the external genitalia are typically female and are greatly hypertrophied as are all other parts of the female genital systems of these specimens

Differentiation of amniote gonads is shown schematically in Figure 8 Some of the details of the diagram are still controversial Not every one agrees that in all mammals or even in most mammals primary sex cords originate quite as diagrammatically as indicated Figure 8 *A* represents the stage of the indifferent gonad when primary sex cords are forming according to the classical scheme and these represent the future tubules of the testis At the next stage (Fig 8 *B*) the germinal epithelium is still present consequently this stage is still bisexual in its potentialities As long as the germinal epithelium is present it is theoretically possible to stimulate a secondary proliferation of sex cords and the formation of a cortical zone (Fig 8 *D*) As soon as the germinal epithelium disappears or becomes atrophic as it does in normal male opossums a day or two after birth (Fig 8 *C*) it is no longer possible to cause the transformation of the gonad into an ovary by treatment with female sex hormone Thus the earlier experiments on opossums were initiated too late to bring about extreme modification of the gonads whereas more recent experiments initiated immediately after birth produce such extreme modification The germinal epithelium may be considered to be an endocrine receptor since it can respond to treatment with female sex hormone by forming an ovarian cortex

Ovaries of genetic females are also modified by the same treatment that transforms testes of genetic males (Burns 1956*b* Fig 10) The ovaries are somewhat reduced in size The cortex is not so well developed as in the normal ovary There is the same reduction in the number of germ cells as in the testes of treated males though in lesser degree The fact that the number of germ cells is also reduced in treated females rules out the possibility that their reduction in treated males is due merely to the inability of a transformed testis to realize the full

and 12) and in places becomes 3 or 4 cells thick It shows signs of proliferating again to form secondary sex cords (cortical cords)

If treatment with estradiol dipropionate begins immediately after birth, the effects are still more striking (see Burns, 1956*b*, Figs 13 and 14) The testis is again greatly retarded Tubules are beginning to degenerate They show vacuolation in some regions of the epithelium The tunic zone is extremely thick, and the persistent germinal epithelium is becoming quite active over the entire surface of the testis Formation of secondary sex cords is indicated They always originate from the germinal epithelium and thus are completely separated from the testicular part of the gonad by the wide fibrous tunic zone There is no continuous basement membrane beneath the germinal epithelium It is constantly interrupted and broken down wherever this kind of proliferation is occurring i e , wherever proliferation of secondary cords is under way In still another specimen (Burns 1956*b* Figs 17 and 18) the cortical cords are represented by great solid clusters of cells There is no sign of germ cells in the cortex The greatly thickened germinal epithelium is still present but there are no germ cells in the germinal epithelium either In such specimens the cortex may constitute two thirds to three quarters of the entire volume of the gland The cortex of the normal ovary of the same age (see Burns 1956*b* Fig 27) possesses cords with the same kind of configuration, but these are packed with germ cells Actually the germinal epithelium of the *normal ovary is less well developed than in the treated embryo* In other words, the germinal epithelium of the ovotestis produced by treatment with estradiol is hypertrophic and this is always the case In several litters treated in this way every male without exception presented this picture

The absence of germ cells has been found to be due to the dosage of hormone used The dosage was approximately 1 or 2 μg of estradiol per day This is a very low dose of hormone in comparison with dosages that have ordinarily been used in such experiments both in placental mammals and in all the earlier experiments with the opossum Yet if the dose is cut to as little as $\frac{1}{8}$ μg per day germ cells appear in the cortex as do rudimentary follicles and even follicles in which the germ cells have entered into the ovocyte stage and are beginning to grow (see Burns 1956*b* Figs 22 and 23 24 and 26) Near the center is all that is left of the original testicular structure of the gonad the thick whitish, non staining material represents the tunic zone Three litters have shown this kind of response In some of these animals the gonads are so completely *transformed that it would be difficult to convince*

potentialities of a normal ovary In other words this particular defect in the gonads of transformed males is not associated with the transformation of sex per se The germinal epithelium of the treated ovaries shows the same hypertrophy that is seen in the germinal epithelium of the transformed testes and this is difficult to interpret In spite of the fact that the germinal epithelium is greatly hypertrophied the *cortex itself is less developed than in a normal ovary and there are* fewer germ cells than in the normal ovary It appears that the hormone actually interferes with the multiplication of primordial germ cells and thus reduces their number

PRICE asked whether with a high dose of estrogen the germ cells are ever completely lacking BURNS replied that in the same litters in which the cortex of the transformed testes is sterile the ovarian cortex is never completely sterile but the number of germ cells is reduced PRICE then inquired whether there are fewer germ cells with a higher dose than with a lower one BURNS replied that this is usually true WEISS asked whether the nuclei are conspicuously larger in the hypertrophied germinal epithelium of treated specimens He suggested that they might be tetraploid nuclei i e that their larger size might be due to failure of cell division BURNS replied that he had never seen any indication to suggest that the nuclei are larger than normal although he had never scrutinized his slides with that possibility in mind It would be necessary to measure a great many nuclei to demonstrate that point

BURNS then commented on the fact mentioned earlier that the cortex of transformed testes contains a number of larger follicles containing young oocytes the nuclei of which distinctly show early prophase changes Such large follicles of this character do not occur in the normal cortex of the same age Thus along with a hypertrophy of the germinal epithelium goes the marked enlargement of a certain number of follicles in the cortex of the transformed testis In each case there is a stimulation direct or indirect over and above the normal growth of these structures

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FIG 8 —Schematic illustration of the process of sex differentiation in the gonads of amniotes showing the various constituent tissues of the embryonic gonad and their future potentialities To induce transformation of the testis by *estradiol dip opionate* in the opossum it is necessary to begin treatment at the tage represented by *B* At this stage the primary sex cords (future seminiferous tubules) have been lai l down but the germinal epithelium is still p esent and well preserved In spite of the genetic male constitution the action of the female hormone at this stage leads to preservation of the germinal epithelium and gradual degenerati n of the male elements When the latter process has reached a certain point the germinal epithelium becomes active and behaves as in *D* Redrawn from Burns (1955 1956*b*)

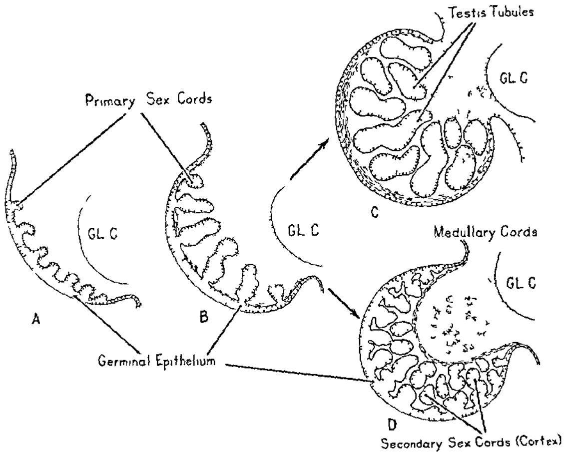


Fig 8.—Legend on facing page

of the freemartin an "experiment of Nature as Lillie called it and that involves a *transformation in the opposite direction* from an ovary into a testis

WEISS asked, *if the pituitary is involved in these sex reversal effects,* how it discriminates between male and female sex hormones BURNS answered that according to the classical concept of Moore and Price (1932) concerning the effects of sex hormones on the pituitary, both types of hormone male and female exert a depressing or inhibitory effect on the gonadotrophic functions of the pituitary Discrimination by the pituitary between male and female hormone is not involved in his experiments since he used only one sex hormone namely estrogen If the pituitary is invoked to explain the results obtained in the experiments under discussion the reasoning might be as follows the hormone by depressing the gonadotrophic function of the pituitary prevents *indirectly* the normal growth and differentiation of the testis components—tubules and interstitium Failure of these elements to develop permits as a release phenomenon the germinal epithelium to survive and ultimately to form a cortex However the suppression of testis differentiation is so prompt in specimens treated immediately after birth and is so extreme that *direct* action of the female hormone on the testis components must be considered In either case, cortical development could still be regarded as a release phenomenon except that the *hypertrophic response of the germinal epithelium and the formation of* precocious follicles are hardly consistent with this interpretation All that can be said is that in the indifferent gonad and in the young developing testis both male and female sex components are present In the testis the male component is represented by the tubular structures and by the interstitium the female component is represented by the persisting germinal epithelium and later on by the cortical cords which it produces It is quite clear that directly or indirectly—and at the present time no decision is possible—estradiol inhibits development of the male components and stimulates development of the female components which of course is the way female hormone should act according to classical concepts

WILLIER questioned Burns about his statement that the germinal epithelium *is a female sex component* He emphasized that either the germinal epithelium can produce the covering of the testis or it can under the influence of female sex hormone produce the female sex component—the cortex He pointed out that this suggests that some sort of switch mechanism may be involved in determining the fate of the germinal epithelium For this reason he does not consider it advisable to

In summary, the treatment of litters of opossums with female hormone causes the following changes in genetic males (1) the most striking effect noted in the earlier stages of treatment is a marked retardation or inhibition of the development of the testis as a whole The development of the testis cords is arrested Later they undergo shrinkage and degeneration to a point where they resemble the vestigial medullary cords of the ovary (2) *The interstitial tissue is greatly repressed* The nuclei appear dense and pyknotic, and ultimately this tissue disappears entirely (3) There is a diminution in the germ cells These three effects are of an inhibitory or repressive character Two effects of a positive nature have been noted (1) the germinal epithelium in treated animals of both sexes is hypertrophic to such an extent that it greatly exceeds in thickness the germinal epithelium of normal ovaries, and (2) a certain number of young follicles in the cortex of transformed testes exhibit precocious growth The large ovocytes in these follicles measure about 25 μ, twice the diameter of the largest follicles in normal ovaries of the same age

PRICE asked Burns whether he visualized the inhibition of the seminiferous tubules as an indirect effect through the pituitary or as a direct effect She suggested that the inhibition of the testicular tubules might be due to the fact that the cortex is developing and inhibiting them BURNS stated that it is impossible to say with any certainty whether the effect of hormone treatment on the seminiferous tubules is direct or whether it is exerted through the agency of the pituitary The simultaneous inhibition of the interstitium which presumably eliminates production of the male hormone in the embryonic testis, could result in the persistence of the germinal epithelium and the development of the cortex Thus development of the germinal epithelium and the cortex could be a release phenomenon due to the lack of inhibition by testicular secretion Merely because of release from inhibition the germinal epithelium might persist for a while and then proliferate secondary cords to form a cortex But that does not explain the hypertrophic effect on the germinal epithelium and certain follicles which represents growth over and above normal Something more than a simple release is involved because the germinal epithelium does not merely survive and grow to the same degree as in the normal ovary it is exceedingly hypertrophied In this case there is a positive stimulation but whether it is direct or indirect in nature is unknown The chief significance of these results is that they are the first instance in any mammal in which the histological differentiation of the gonad has been reversed experimentally The only similar case on record is that

(1952) demonstrated that the approximation of an embryonic testis to a bit of ovarian tissue in vitro results in formation of a cortex on the testis In this case the receptor is responding in the absence of the rest of the organism, including the anterior pituitary SEGAL stated that sex reversal can be brought about similarly in hypophysectomized larvae of amphibians

BURNS then suggested that the only reason for suspecting the pituitary at all is in comparison with the events after unilateral removal of an ovary in many animals The number of maturing follicles in the remaining ovary essentially doubles under these circumstances And if the remaining ovary is fragmented and only a portion of it is left, the number of maturing follicles in that fragment of an ovary again increases A kind of compensatory phenomenon takes place which has been explained on the basis of gonadotrophic effects on the remaining ovary or piece of ovary The precocious growth of a few follicles in the testicular cortex is essentially the same kind of compensation as that which occurs after partial ovariectomy in mammals He then stated that the question of the intervention of the pituitary in any differential way in sexual differentiation has scarcely been raised It seems to have no importance except that it may influence quantitatively the growth of structures later on It is not qualitatively involved in the phenomena of sex differentiation WEISS was anxious to have this point emphasized for the record

PRICE then inquired whether estrogen treatment stops migration of germ cells from the extra gonadal areas BURNS answered that this possibility has not been explored He stated that nothing is known about the primordial germ cells of the opossum before they are in the gonad Whether the sterility of the cortex in the earlier experiments could be attributed to an early arrest of germ cell movement seems doubtful since the testicular part of these gonads is not sterile WILLIER inquired whether the germ cells of the opossum are already in the germinal epithelium at the earliest normal stages examined BURNS answered that they are in the gonad but not in the germinal epithelium They are always beneath the germinal epithelium in the stromal part of the gonad He has seen no evidence that they come from the germinal epithelium although his observations are not conclusive on this point

MARTINOVITCH then asked Burns whether his interpretation of the action of the hormone on the germ cell tract is that the hormone may inhibit cellular division BURNS replied that this is all he can think of The number of germ cells is greatly reduced It is reduced in both the testis and the ovary The only treatment has been the administration of

refer to the germinal epithelium as a female sex component BURNS agreed that in early stages of gonad formation (Fig 8, *A*) the germinal epithelium cannot be called a female sex component because it is producing the medullary *cords, which are the testicular part of the gonad* However, experimental treatment is begun at the stage illustrated by Figure 8 *B* At this stage the gonad is still bipotential because the testicular elements have been laid down and the germinal epithelium is still *present From this stage on, the germinal epithelium can do only the* two things indicated in Figure 8, *C* and *D* it can atrophy to form the covering membrane of the testis or it can continue to proliferate and form a cortex And in that sense, *at this stage of development* the germinal epithelium when it is present must be regarded as a component of the gonad which has female potentialities and never male

KOLLROS asked whether it is possible to treat the gonad locally with female hormone Such a treatment would eliminate the possibility of intervention by the pituitary, or at least presumably would do so BURNS answered that this would be difficult to do for technical reasons, at least in vivo KOLLROS then illustrated how his laboratory makes use of thyroxin pellets to produce localized effects If such a pellet is placed just under the skin of a tadpole, an effect on the skin glands is evident (see Kollros and Kaltenbach 1952 Fig 5 *a*) Just above the pellet are large glands and molting of the skin A little to one side appear very substantial glands without superficial molting Far to the other side, only gland nests appear The distance in the tadpole over which the local effect may be detected is perhaps 1 mm or maybe 1 5 mm A similar effect can be shown if the pellet is placed immediately adjacent to the area of the skin window where the front legs ordinarily emerge (see Kaltenbach 1953*c* Pl 1) On the side containing the pellet there is a rupture of the window even though the limbs are in a very embryonic condition On the other side which received a control pellet of cholesterol there is no rupture in fact there is still a very thick opercular wall there Because such local effects can be produced in tadpoles the thought suggested itself that pellets of estradiol placed next to the testis might likewise have specific local effects thereby providing one way of analyzing whether there is an influence directly on the gonad or only indirectly through the pituitary gland

Evidence was then introduced by WILLIER which makes it seem unlikely that the pituitary is involved in transformation of the gonad First, he commented on the fact that Wolff (1937) has demonstrated that sex reversal can be produced just as readily in pituitaryless embryos as in intact embryos Second he recalled that Wolff and Haffen

rather old story which has been in the literature for many years The situation in the opossum does not differ significantly from that in half a dozen other mammals in which the mother has been treated with hormone during pregnancy The genital tracts of the young are rather completely transformed in many cases In general the effects of the hormones are fairly specific Female hormones stimulate development of Mullerian ducts and inhibit Wolffian ducts, and vice versa An exception to this last statement must be made in the case of the mammals, in which testosterone and its compounds do not inhibit the Mullerian ducts *except partially in one or two species, Burns (1955) should be con*sulted for details In the opossum for example only the vaginal part of the Mullerian duct is suppressed Opossums which received estradiol treatment possess greatly hypertrophied external genital structures of the female type Estradiol completely inhibits development of the prostates in young, treated males All derivatives of the Mullerian ducts are greatly hypertrophied in both sexes following treatment with estradiol whereas the Wolffian ducts are unmodified and appear as in controls The hormones appear to be acting directly on the individual primordia and the effects are specific if care is taken to control the dosage Paradoxical effects are encountered with exceedingly large dosages especially with androgens which in higher concentrations stimulate Mullerian duct derivatives Lower dosages do not cause such paradoxical effects WILLIER emphasized that such results indicate that there are latent differences in response based on pre existing heterogeneity The sex ducts respond in a differential manner to male or female sex hormones depending on their inherent constitution

MUHLBOCK then presented some of his experiments on granulosa cell tumors in mice which show how sensitive the cells are to different dosages of hormones (Van Nie and Muhlbock 1956) *He stated that* after transplantation most granulosa cell tumors grow better in males than in females In orchidectomized males the rate of growth decreases to the level of growth in females The castration effect in males can be *prevented by androgenic hormones It is striking that estrogens and* progesterone exert the same accelerating effect as do androgens on the growth of these tumors A more detailed study by Van Nie (1957) demonstrated that some of these tumors grow only at a very distinct hormone level (*Fig 9*) *With low doses as with very high doses* the growth of the tumors is not optimal Discontinuation of hormone application causes a very remarkable regression of the tumors It is striking that after transplantation the tumor cells may lie dormant in the sub cutaneous tissue for a long time and only begin to grow after stimula

the female hormone Consequently, this hormone must, in some way, be responsible for the reduction MARTINOVITCH then asked whether there is any evidence of the possibility that the hormone may exert a direct injury effect on the germ cells BURNS answered that this might be the explanation, although he knows of no other evidence to show that exces sive hormone directly injures germ cells The reduction in number of germ cells appears to occur before the spermatocyte or the oocyte stages It is the number of gonia that is reduced Thus whatever the effect of the hormone it is exerted very early

WEISS then inquired whether treatment at the same stage with a whole range of dosages of testosterone from zero to shock doses would give the same effect BURNS answered that he did not know, since up to now there are no experiments in which testosterone was given at the early stages at which an effect on the germ cells is noted KOLLROS sug gested that the switch mechanism insofar as the germinal epithelium is concerned, i e whether it will form cortex of the female type or simply the covering of the testis, may be a *direct* effect of the hormone treat ment, whereas the number of germ cells produced may be influenced *in directly* through the pituitary WEISS considered this to be an oversim plification of the situation He suggested that the pituitary may some how be involved by setting the level of the response of the gonad tissue without determining which direction it will take the chemical configu ration of the hormone administered may actually determine the direc tion of development of the receptor BURNS took this occasion to empha size that an analysis of the way in which the female hormone produces its effects would require an almost unlimited number of experiments This would be exceedingly difficult with opossums because it is ex tremely difficult to obtain litters young enough to produce the transfor mation of the testis The number of litters born at a stage when the ger minal epithelium is till present is only a very small proportion of the total litters available Thus there is considerable variation in the condi tion of the gonads at the moment young opossums are born The experi menter must always wait for litters in just the right stage of develop ment at the time of birth and this means that studies of this type cannot progress as rapidly as desirable

DR WILLIER called attention to the gonoducts as endocrine receptors and asked Burns to comment briefly on the effect of sex hormones on the various sex ducts Is the hormone selective in its action on the sex ducts? Is this effect direct or indirect? If the testis is caused to trans form into an ovary or into an ovotestis are there simultaneous changes in the female direction in the gonoducts? BURNS stated that this is a

tion with hormones Moreover if the tumor undergoes a certain morphologic change it is no longer dependent on the hormone It somehow or other loses its ability to respond selectively It can no longer be suppressed with high dosages of the hormone This effect is independent of the pituitary gland, since the same results are obtained when the experiments are performed on hypophysectomized animals SCHNEIDERMAN asked whether the tumor had lost the ability to respond or whether it had gained the ability to be independent When a cell differentiates it certainly loses the ability to produce certain substances and in the process gains nutritional requirements WEISS put it this way If hydrogen and chlorine are combined to form hydrochloric acid they lose the properties of the hydrogen and the chlorine but they gain the properties of the acid!

SEGAL next presented an example of Nature s experiment in sex reversal in the mammalian gonad He illustrated a testicular biopsy which was taken from a human genetic female as proved by the sex chromatin test (Segal and Nelson 1957) This genetic female developed infertile testes and the complete secondary sex apparatus of a male This case and all others of this so called Klinefelter syndrome involve individuals who are raised as men and who frequently marry as males In some cases spermatogenesis is complete in some tubules This condition must certainly have an embryonic origin with the indifferent embryonic gonad differentiating in a direction opposite from its genetic constitution The normally dominant embryonic gonadal cortex of this genetic female has been suppressed in favor of the development of the medulla

WILLIER then reviewed experiments performed in his laboratory much earlier involving administration of sex hormones to chick embryos (Willier 1939 1942) In genetic males there is an incipient ovarian cortex on the left gonad which is usually lacking on the right side although occasionally a little bit of ovarian cortex is present on the right side also As long as this incipient ovarian cortex is present when introduction of female hormone occurs it can respond by developing into a beautiful and typical ovarian cortex The two sides of the body differ with respect to the time that the incipient cortex disappears If differentiation proceeds in the male direction the primary sex cords become the seminiferous tubules and certain ducts persist to become passageways for escape of spermatozoa The Mullerian ducts disappear If differentiation proceeds in the female direction an ovarian cortex forms on the left side The primary sex cords remain on the left but they assume a different form than they do in the male they hollow out instead

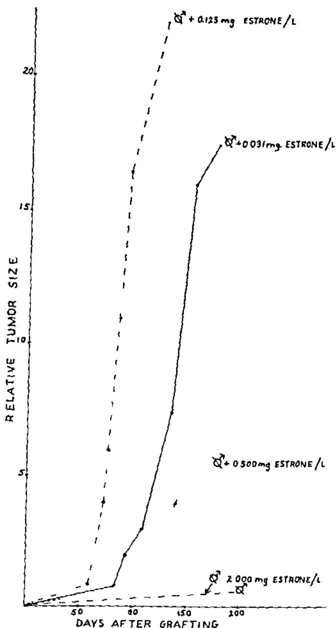


FIG 9 —Influence of different doses of estrone in drinking water on the growth of a transplanted ovarian tumor Redrawn from Van Nie (1957)

The Wolffian ducts and certain mesonephric tubules which begin to hy pertrophy with low dosages become enormously swollen when 1 mg or more of hormone is given Thus in these experiments the receptors re spond to sex hormones selectively Male components respond to male hormone whether they are present in genetic males or in genetic fe males, and female components respond to female hormone whether they are present in genetic males or in genetic females Thus as in the case of mammals there are latent differences in response based on pre existing heterogeneity The sex components are already tuned, so to speak to re spond to a specific kind of hormone The same kinds of changes can be brought about by injections of sex hormones into duck embryos

Domm (1939) and others have shown that when the left ovary of a young bird is removed after hatching, the right ovary which is poten tially a testis, actually transforms into a testis Similarly, the left ovar ian medulla transforms into a testis after removal of its cortex Thus the ovarian cortex seems to be exerting an inhibitory effect on the ex pression of the male components of the ovary WEISS asked in connec tion with what had been said earlier about differences in heterozygous and homozygous sexes in birds, as compared with mammals whether there is any evidence in mammals that the *male* component of the gon ads exerts an inhibitory influence on the cortical component In other words is the situation just the opposite from that found in birds? SEGAL stated that the case of human sex reversal in the Klinefelter syndrome, presented earlier, illustrates exactly this condition The embryological aspects of this situation have been completely discussed in recent papers by Segal and Nelson (1957) and by Witschi Nelson and Segal (1957) BURNS stated that in the opossum there is no experimental evidence on this point as yet although as he mentioned earlier it is possible to ex plain the development of the cortex on the transformed testes of opos sums treated with female sex hormone in terms of a release from an in hibition normally exercised by the medullary component WILLIER stated that the freemartin might be a case of this sort because the left ovarian cortex failed to develop in most of Lillie s cases Bissonnette (1924) however had some cases in which there was a little ovarian cor tex present Under these circumstances an ovotestis was produced Whether an incipient ovarian cortex is present in cattle has not been demonstrated however an incipient ovarian cortex has been demon strated in certain mammals like the cat BURNS emphasized that the germinal epithelium itself at a certain stage represents an incipient cor tex i e during the indifferent stage of the gonad WEISS inquired whether anyone has transplanted a young male gonad into the early

of remaining solid The oviduct develops from the Mullerian duct on the left side only, it disappears on the right side, with the exception of a small rudiment Thus the embryo has a bisexual organization at first, with both male and female components, just as in the mammal

Sex differentiation can be modified by the introduction of sex hormones Even though the dosage is carefully controlled it is impossible to determine the exact dosage actually reaching the embryo hence the results are somewhat variable If female sex hormone is added to genetic males the left testis is altered in form and structure it becomes a flattened, ovary like body consisting of both ovarian and testicular tissue (ovotestis) In extreme cases it is practically indistinguishable from a normal left ovary (see Willier, 1939, Figs 28 and 31) Near the hilus is typical testicular tissue More centrally located is medullary tissue but the sex cords in this location are hollowed out to form the female pattern of the male sex component A thick ovarian cortex occurs at the surface and contains many germ cells If female sex hormone is administered after the ninth day when the incipient ovarian cortex has disappeared no reversal effects can be obtained In such experiments the original sex of the treated embryo can be determined because the fertile eggs come from a cross between two breeds of chickens in which all the male chicks have a patch of white feathers on the top of the head whereas the female chicks have black plumage over the head These plumage characteristics are independent of sex hormones The right testis of treated males remains unchanged except in those cases exhibiting complete or nearly complete reversal of the left testis, then it is reduced in size flattened and contains ovarian medullary tissue its testicular nature is never lost The Mullerian ducts persist on both sides, either partially or completely depending on the dosages given With very large dosages they become hypertrophied There is no effect on the Wolffian ducts In the normal male the testes are cylindrical in outline and the Mullerian ducts are absent (see Willier 1939 Fig 29)

If male sex hormone is added to genetic females the form and structure of the ovaries are altered (Willier 1939 Figs 30 and 33) Each ovary, and especially the right assumes the form of a testis The ovarian cortex on the left gonad thins out and degenerates while the ovarian medulla hypertrophies and transforms into testicular tissue at the hilus The medulla of the right ovary hypertrophies and transforms into testicular sex cords It is much more difficult to bring about the reversal of the sex components of the female gonad than of the male gonad Oviducts exhibit various grades of retrogressive changes the left one often undergoing reduction to a short rudimentary portion containing a lumen

generation in explants taken from the left ovary, and especially in cultures made from its caudal pole Cordlike proliferations develop in which numerous oogonia and oocytes in all stages of meiotic prophase are to be seen Such differential behavior of explants taken from left and right ovaries also exists when donors are 4 , 5 , or 6 month fetuses These observations seem to indicate some kind of inequality in the regenerative capacities of organ fragments isolated from the two sides of the body PRICE inquired how many explants were involved GAILLARD answered approximately 45 of each

WILLIER then pointed out in this connection that there is evidence that in monotremes the left ovary is the one that produces the eggs Furthermore in the case of the freemartin, the changes differ on the right and left sides BURNS commented that he had not noticed distinct lateral differences between the two sides in the reaction of sex primordia in the opossum but that he was not prepared to state that differences of this nature would not be found if precise measurements were made Certainly if they exist they are not so conspicuous as in birds SCHOTTE then asked whether there is any evidence that ovarian tumors occur more frequently on the left than on the right side in humans MUHLBOCK replied that in their experiments they often see a tumor on one side only, but that there is no difference in frequency between left and right sides

WEISS then stated that it was his feeling that a point had been reached in the discussion where special attention should be given to the factors involved in the establishment of the latent differences in response of the receptors Development begins of course, with a zygote, which is genetically male or genetically female, and as development progresses various embryonic rudiments arise whose cells are all genetically male or genetically female When hormones are added to the developing system either from the outside (exogenous hormones) or from the inside (endogenous hormones) genetically male rudiments respond differently at least quantitatively from genetically female rudiments He raised several questions about the progress of development of the rudiments Do they develop as completely closed systems up to the moment when they are suddenly confronted with hormones for the first time? And when they are first exposed to hormones are they then changed in some way once and for all such that they are not influenced in any way thereafter by the presence of these hormones? The original difference in genetic composition of zygotic male and zygotic females may be detectable in early stages by differences in sex chromatin in the nucleolus etc But the different reactions of receptors of zygotic males and zygotic females

cortex of an ovary to see whether there is a local inhibitory effect BURNS *replied that it would be very difficult to do technically but that* doubtless a clever operator like Dr Jost could do the operation WILLIER added that Wolff has been able in some of his experiments to produce an ovarian cortex on the right ovary, provided that the treatment begins *early enough Typically the right ovary lacks an ovarian cortex, but oc*casionally little patches of ovarian cortex are present on the right gonad, and these can then respond to treatment with female sex hormones

PRICE inquired whether there is any explanation of the asymmetry in the avian reproductive system WILLIER replied that this is but part of the fundamental asymmetry of the chick embryo Even in earlier stages i e, in primitive streak and head process stages there are differences in the capacity of the two sides of the body to produce an organ such as the eye in isolates transplanted to the chorio allantoic membrane If the transverse eye forming region is cut into three pieces (right, median and left) the capacity of the right piece for formation of an eye is lower than the capacity of the left piece The median piece is more or less intermediate between the two lateral pieces In the case of every organ forming system that has been examined, there is a greater tendency to produce more tissue and more normal tissue, in grafts of pieces taken from the left side of the body WEISS mentioned that the same thing is true of amphibians and sea urchins WILLIER suggested that we may be wrong in teaching students that organisms are bilaterally symmetrical actually, they are bilaterally asymmetrical WEISS commented that all this really amounts to is that one side of the body is ahead of the other it gets a head start in development and thus is a little older than the other half BURNS also mentioned the fact that there is an asymmetry *in the adult organism that is not confined to the reproductive tract* For example the aortic arch in birds is on the right side and not on the left as in mammals and there are other very marked asymmetries in the adult

In relation to this topic of asymmetry GAILLARD *next commented* briefly on ome differences in the behavior of explants derived from the right and left ovaries of man When organ cultures of the peripheral part of the cortex (including the germinal epithelium) are made from the ovaries *taken from a* $3\frac{1}{2}$ *month human fetus* the explants taken from the right ovary begin to degenerate after some days Complete disorganization of the typical ovarian structure occurs and within 1 week it is hardly possible to recognize the tissue as a derivative of the ovary *complete degeneration may occur* By contrast there is a complete re

It is absurd therefore to inquire whether a particular response is de termined hormonally or genetically Even if it appears superficially to be determined hormonally the hormone source is determined by the genome plus the embryonic history that is behind it when its product is first encountered Hormone action is probably not a one shot operation, the agent involved more likely operates at different times repeatedly, producing different results depending on the time at which it works It may change a given system in a given way at one moment but it likely exerts its effects again and again on the system as time goes on

SCHNEIDERMAN commented that it is probably the precipitousness of things like metamorphosis that causes us to think too much of the trig gering action of hormones rather than of a sustained and repetitive ac tion at different stages of maturity He mentioned that in the case of metamorphosis of insects it makes a great deal of difference whether a big dose of hormone is given at one time or whether a little bit is given over a long time quite different types of organisms are produced BURNS pointed out that at various points along any given chain of in teractions that is involved in the development of any receptor some of its final characteristics can be influenced with hormones, but others can not The system is in constant transformation But BURNS also empha sized that at given moments in development certain irreversible changes can and do occur almost instantaneously perhaps not in a matter of minutes or even of hours but within a relatively short span of time He recalled the situation in the opossum testis which he had discussed earlier and illustrated a section in which the germinal epithelium on the surface of the testis had separated from the primary sex cords (see Burns 1956*b* Fig 5) He emphasized that this is about 24 hours after the stage at which hormone injections were made By this stage it is quite impossible to restore any activity of the germinal epithelium Thus within 24 hours the germinal epithelium has lost its capacity to respond to estrogen and no treatment will cause any further development of the cortex or even any hypertrophy of the germinal epithelium WILLIER stated that the same thing is true of the left testis of the chick It rather suddenly reaches a stage where it is no longer responsive BURNS sug gested that there is a similar critical period for the prostatic buds al though perhaps not so brief WEISS stated that he wished only to em phasize that the developing system loses what it does as a result of the environment it has been exposed to during its development prior to that time rather than exclusively as the result of an innate change of its own

SEGAL then reported on the work and interpretations of Chang (Chang 1955 Chang and Witschi 1955*b*) which provide an example

must be conditioned by much more than the original genetic differences of the cells present in the receptors The cells of each receptor as they develop are surrounded and influenced by a male or a female environ ment consisting of neighboring male or female cells and their products It is well known from experimental embryology that many cellular in teractions play back and forth in a complex fashion between the vari ous organ rudiments derived from the zygote The reactions between the *organ rudiments of a male zygote ought to be somewhat different from* the reactions between the organ rudiments of a female zygote, whether we can detect them or not When the establishment of heterogeneity in receptors is discussed, what should be considered is the sum total not only of the initial differences in genetic composition but of all the dif ferent interactions within male and female individuals that take place during their developmental history If this is true, then the underlying conditions for quantitatively different reactions of male and female rudiments to the same hormonal conditions originate progressively with time During development of the pituitary glands of genetic males and *genetic females there is probably never a stage when the cells are ex* actly identical in the two sexes in their response to hormones They are different when they begin to develop, but they also respond differently to influences from adjacent organs as they develop, and thus they be come increasingly different from one another in a progressive way Whether reactive capacities are established progressively in this way needs to be tested and, unless the problems are posed in this form they will not be tested When something is added from the outside—for ex ample an exogenous hormone—it does not act from scratch, it does not operate on a system which has no history and has just been waiting un *changed for testosterone or stilbestrol or what not to be added* What ever is done experimentally to the organism has to be rated against the background of the previous history of the reacting embryonic structures and this history has not been immune from tissue interactions Instead of talking about an exclusive monopoly of a particular glandular dis charge in the regulation of an otherwise indifferent and ignorant sys tem the possibility should be considered that innumerable agents of similar nature *have been acting on that system throughout its develop* mental history although in a less dramatic way Thus the inherent characteristics of a later stage are more than the genetic constitution received by the zygote they are the products of a historical course of interactions between the original genome and its environment It is rare rather than common that such sharply defined reaction times are en countered as those characteristic of the phenomena of metamorphosis

males and females Even though they do not come from the ovaries and testes and even though removal of the gonads and cross transplantation experiments have no effect at all, this does not mean that they are not produced at other sites

MUHLBOCK then asked whether anything is known about the influence of the age of the parents on the development of the young in mammals He stated that he has some evidence that the age of the mother exerts an influence on the development of tumors in mice (Muhlbock 1956) WEISS referred him to the conference on the prenatal influences in mammalian development that had already been held

of the concept developed by Weiss, namely, that an organ system reacts in accordance with and as a result of its entire past history, including its genotype In *Xenopus* the male is homozygous (XX), whereas the female is heterozygous (XY) Estrogen added to the aquarium water containing *Xenopus* larvae causes feminization of genetic males Andro gens have no effect on genetic females Thus in the latter case a zygote starting with the XY chromosome composition undergoes a develop mental history which precludes a possible interference with sex differ entiation by the administration of androgens XX females produced by the treatment of genetic males with estrogen can be mated with normal XX males The result of such a cross is obviously 100 per cent larvae of the XX genotype i e , genetic males If, during the third week in devel opment such larvae are treated for a 3 day period with estrogens they can be sex reversed to XX females and could later be used in matings as functional females If however, following this short but feminizing treatment with estrogens, these larvae are subjected to the administra tion of androgen the resultant gonad is of the testicular type In other words it is possible to bring about an androgen induced masculinization of the gonad of *Xenopus* larvae if the ovary bearing larva is of the *ho mozygous* genotype If it is of the *heterozygous* type, as under normal circumstances androgen has no such effect This difference in original genotype results in a different potentiality in the receptor organ to re spond to the hormonal stimulus In *Rana* the homozygous female can be masculinized with androgens In *Ambystoma* the heterozygous female like *Xenopus* cannot be sex reversed with androgens Such observations led Chang to postulate that there exists a possible relationship between female homozygosity and a positive masculinizing influence of androgen Thus the genetic background is carried through the lives of these ani mals, and it takes an ingenious experimental design to bring it out WEISS then inquired whether it is generally true that the homozygous sex has the greater inertia so to speak BURNS answered that as far as castration experiments in birds and mammals are concerned the cas trate type in birds closely resembles the normal male (XX) whereas the castrate type in mammals closely resembles the normal female (XX) In *Xenopus* according to Schotte the castrate closely resembles the female (XY)

SCHNEIDERMAN then pointed out that insects have no sex hormones as far as is known The insect sex organs are sex limited from the ear liest stages of their development and cannot be modified by transplan tation WEISS stated that all that can be said from such experiments is that if sex hormones exist in insects the same ones are present in both

traperitoneal administration of diphtheria antitoxin, the animals die from diphtheria intoxication But in the periphery of the body the toxin is neutralized Under the conditions of this experiment the adrenal cortex shows only the physiologic signs of ACTH stimulation (increased number of mitoses increased nuclear volume in all three zones, and the so called progressive transformation) but no hemorrhage and no necrosis

Diphtheria toxin is distributed over the entire body after injection But normally other organs such as the ovaries and the testes do not respond even to very high doses of the toxin However, pretreatment of infant animals with gonadotrophins followed by the injection of only 2 M.L D of the toxin results in a very pronounced hemorrhagic necrosis of the ovaries or the testes Even more instructive is the observation that hypophysectomized and castrated female guinea pigs previously treated with estradiol show after administration of diphtheria toxin a severe hemorrhagic necrosis of the endometrium but no lesions of the adrenal cortex Without treatment with estradiol even very high doses of the toxin are ineffective on the mucosa of the uterus

The specific action of other kinds of bacterial toxins differs from that of diphtheria toxin As far as their effect on the adrenal cortex is con cerned three types of bacterial products can be recognized (Table 2) (1) Botulinus toxin acts on the adrenal gland only as other stressors do Its effect on the adrenals is purely non specific There is no specific tissue damaging action Therefore the adrenal shows no destructive lesions (2) Diphtheria toxin possesses a tissue damaging action that is strictly related to the stimulation of the adrenals by ACTH (3) Meningococcal toxin gives two kinds of action (*a*) a non specific ac tion resulting in release of ACTH by the pituitary and (*b*) a specific tissue damaging effect which contrary to the diphtheria toxin is not principally related to stimulation of the gland by ACTH Hemorrhage and necrosis occur even in the absence of the pituitary For details consult Tonutti (1953 1956)

WEISS inquired whether the action of hypophysectomy is due to the fact that excitability of the tissues normally increases in the absence of the pituitary He suggested that it might also be due to the fact that removal of the hypophysis brings other endocrine organs into compensa tory operation TONUTTI answered that it is the absence of ACTH per se which is responsible becau e normal behavior of the tissue can be com pletely restored by substitution with ACTH The gonads adrenals, and thyroids are inactive after hypophysectomy No other glands have a similar action WEISS then inquired whether diphtheria toxin has an

VIII

Conditioning Effects on End Organs

According to WEISS, conditioning is a non overt act it is an action on an organic system that leaves a residue which is not immediately detectable Any subsequent action will have been modified by the previous activity of the so called conditioning agent In the case of a nerve, conditioning changes the excitatory state or the threshold of the system This results in an expressed or overt action when another factor (stimulus) is introduced at a lower or higher dose or for a longer or shorter exposure

TONUTTI then illustrated the conditioning effect of hormones on the destructive action of bacterial toxins against tissues (Tonutti, 1953) It is well known that diphtheria toxin causes practically complete destruction of the adrenal gland within 24 hours if administered at a dose of 5 M L D The organ loses its yellow color and becomes cherry red Histologic examination reveals a severe hemorrhagic reaction and severe necrosis In the hypophysectomized guinea pig the adrenal remains yellow after inoculation with the same dose of diphtheria toxin histologically, there is no vascular reaction and no necrosis Hypophysectomy therefore completely protects the adrenal tissue against the destructive action of diphtheria toxin or in other words adrenal tissue loses its responsiveness to diphtheria toxin after hypophysectomy If hypophysectomized guinea pigs are treated with ACTH and are then injected with diphtheria toxin the responsiveness of the adrenal tissue to the toxin reappears This seems to be definite proof that the destructive action of diphtheria toxin on the adrenal is conditioned by ACTH

In the normal animal diphtheria toxin has two actions (1) a non specific action which results in the release of ACTH by the pituitary as a result of which there is a physiologic stimulation of the adrenal by ACTH, and (2) a specific action which leads to bleeding and necrosis in the adrenal but only if the adrenal tissue is stimulated by ACTH The non specific action can be separated from the specific tissue damaging effect of the toxin by the following experiment If diphtheria toxin is injected into the brain of animals previously immunized by in

of action of the stromal component of organs TONUTTI stated that usually the capillaries in normal adrenals are not very large and are not readily seen after treatment with diphtheria toxin they become very much enlarged They suffer stasis and some exudation takes place WEISS suggested that the occurrence of stasis suggests the existence of a local constriction somewhere The capillary bed has clamped down Thus there must be an action on the capillary wall WILLIER agreed that this is probably so because the capillary wall of the adrenal gland is no doubt different from the capillary wall of any other endocrine gland and consequently can react in a selective way It is likely that the capillary part of each organ assumes gradually during its development the same type of specificity as the other components of that organ TONUTTI then pointed out that there is even selectivity within the adrenal in this respect The capillaries of the medulla of normal animals react against diphtheria toxin even earlier than those of the cortex In the hypophysectomized animals the capillary reaction is restricted sharply to the capillaries of the medulla WILLIER then inquired concerning the direction of blood flow in the adrenal TONUTTI answered that it flows from the capsule to the medulla He also emphasized that it is astonishing that the medullary capillaries which are continuations of the capillaries of the cortex react after hypophysectomy whereas the latter do not The two sets of capillaries must be different in their reactive capacities

WEISS then asked about the vascular reaction of the comb of the fowl to hormone treatment WILLIER indicated that it i a similar story except that a mucoid type of connective tissue is involved WEISS then asked whether in this case the primary reaction to the hormone treatment is in the capillary bed or in the mucoid tissue TONUTTI stated that it is in the capillaries This means that the primary reaction is the dilatation of the capillaries and the secondary reaction is a deposition of ground substance He also pointed out another fine example namely the sexual skin of the baboon (Bachman Collip and Selye 1936) This is an end organ for estradiol When it is transplanted to another region of the body the transplanted sexual skin also becomes red But flank skin transplanted to the sex region of the baboon remains pale

PRICE then inquired whether ACTH acts directly on the walls of the capillaries or whether it simply stimulates production of corticoids which affect the capillary wall The hemorrhage might well result from the effects of the cortisone produced in response to ACTH TONUTTI answered that this is not the case If animals are prepared with cortisone for 3 weeks causing suppression of ACTH secretion the adrenals fail

effect on mitotic activity TONUTTI answered that when bleeding occurs in the adrenal gland after treatment of the animal with diphtheria toxin, mitosis stops immediately There are no mitoses in any animal in which the adrenal is hemorrhagic unless there are only small spots of hemorrhagic necrosis If the destructive action of the toxin is absent there are great numbers of mitoses

TABLE 2*

EFFECTS OF THREE TYPES OF BACTERIAL TOXINS ON THE ADRENAL CORTEX

			NON SPECIFIC STRESS ACTION ON ADRENALS		SPECIFIC NOCUOUS ACTION ON ADRENAL TISSUE	
			Depletion of Cholesterol Esters	Morphokinetic Action	Hemorrhage	Necrosis
I	Botulinus toxin	Normal	ruled	ruled		
		Hypophysectomized				
II	Diphtheria toxin	Normal	ruled	ruled	ruled	ruled
		Hypophysectomized				
III	Coli lipopolysaccharide or meningococcal toxin	Normal	ruled	ruled	ruled	ruled
		Hypophysectomized			ruled	ruled

Blank = negative ruled = positive From Tonutti (1953)

WEISS *then asked whether there is any information about the point* of attack of ACTH in this case What does it sensitize? Does the endothelium of the capillaries become fragile or what does happen? TONUTTI answered that the ACTH acts on all components of the adrenal cortexes capillary walls interstitial tissue and parenchymal cells He considers the adrenal tissue to respond as a whole WEISS expanded his question into a discussion He inquired to what extent this is always true of composite organs where there is always an epithelial component a stroma and a vascular bed in addition to nerves Does the stroma or the vascular bed react selectively? He recalled that the earlier discussion of embryological phenomena placed increased emphasis on the specificity

sectomy and that of course, the capillaries of this region degenerate

WEISS summarized the discussion by stating that (*a*) some of the hormonal effects are not directly visible but provide fluctuating backgrounds against which each individual agent must be rated and (*b*) the point of attack of these may be not only on the specific glandular tissue or the most conspicuous part of the organ but on the capillary network as well

He then asked what is known about the stroma of an organ in any response situation The stroma does change, but does it do so in an unspecific way? Is it simply the result of the alterations of the specific epithelial tissues and/or vascular tissues or is the stroma itself differently disposed in different parts of the body in its response to hormonal influences? SEGAL called attention to the effects of estrogen on the prostates Some investigators have reported that it is the increase in the stromal component of the rat prostate after estrogen treatment that actually causes an increase in the weight of the gland The weight of the gland decreases in pituitaryless rats and then increases following administration of estrogen Histologic examination shows this increase to be in the stroma and not in the glandular elements of the prostate Burns suggested that the reaction of the stromal tissues is not limited to the prostate The connective tissues and sheaths of the entire reproductive tract show this kind of hypertrophy in the presence of estrogen The greatly swollen external genitalia of the young opossums treated with estrogen are due to the reaction of the stromal elements The sheath around the sinus and around the erectile bodies is enormously hypertrophied in the presence of estrogen The connective tissue of the uterine wall reacts in the ame way and of course you get the same reaction in the prostate because the prostate stroma is derived from the sheath of the sinus SEGAL emphasized that in the same animal in which a proliferation of the stroma of the urogenital system occurs there is no parallel proliferation of the subcutaneous stromal elements WEISS inquired whether the response of subcutaneous connective tissue in endocrine obesity is a case in point This would be a localized response to hormonal imbalance

GAILLARD reviewed experiments involving organ culture Slices of the uterus of 3 week old mice provided with 0 005 I U of estrone undergo proliferation exclusively in the connective tissue of the endometrium the addition of 0 050 I U of estrone leads to a proliferation of smooth muscle cells If o called pure strains of periosteal fibroblasts and of heart fibroblasts are cultured and are treated with crystalline growth hormone the heart fibroblasts react optimally with mitosis at a concen

to react just as in the case of hypophysectomy Thus cortisone cannot be the conditioning factor for the lesion of the capillaries following treatment with the toxin

WEISS returned the discussion to the experiments involving baboon skin Where do the capillaries of the transplanted skin come from? If they arise from the local area where the graft is placed, they must have acquired their specific response from the transplanted epithelial com ponent But is it not equally possible that the capillary bed is brought along with the transplant? GAILLARD mentioned that in the work of Merwin and Algire (1956) the capillaries sprout out from both the graft and the host WEISS agreed and stated that he believes that a lot of the incompatibility reactions between graft and host are due to the incompatibility of the two types of vessels in the vascular mosaic Some of the terminal roots of the blood vessels come from the graft MARTINOVITCH mentioned that in heterotransplants (rat to cat) it is only the vessels of capillary size that enter the transplant He never observed a vessel of the size of an artery or vein to penetrate the graft (Martinovitch, 1956) In homotransplants of the rat adrenal gland a secondary inner capsule appears if the host is hypophysectomized If the hosts are then treated with ACTH the secondary capsule disappears as the cortex begins to regenerate apparently the tissue of the capsule is used up in building new vessels (Martinovitch 1955) WEISS then stated that, even if the capillaries come from the transplant, they still have to acquire their specificity earlier in ontogeny as part of mosaic formation

MARTINOVITCH then inquired whether Dr Tonutti had ever seen the collapse of blood vessels in the adrenal following hypophysectomy TONUTTI replied that the whole adrenal shrinks MARTINOVITCH men tioned that hemorrhages are a fairly common occurrence in ocular adrenal grafts They are usually followed by a reduction in the size of the graft which may indicate the obliteration of some of the vessels WEISS asked Martinovitch whether the point he was trying to make is that even simple hypophysectomy can cause a partial collapse which would then be accentuated by diphtheria toxin However TONUTTI again emphasized that there is no reaction in the hypophysectomized animal MARTINOVITCH added that extravasation preceded by distention often takes place in grafts following hypophysectomy of the host The fact that the inner capsule comes into sight after hypophysectomy and then disappears after ACTH is added to the hypophysectomized host indicates that some of the vessels undergo degeneration at the time of hypophysectomy just as they do in vitro TONUTTI stated that the whole inner layer of the adrenal undergoes involution after hypophy

sectomy and that of course, the capillaries of this region degenerate

WEISS summarized the discussion by stating that (*a*) some of the hormonal effects are not directly visible but provide fluctuating backgrounds against which each individual agent must be rated and (*b*) the point of attack of these may be not only on the specific glandular tissue or the most conspicuous part of the organ but on the capillary network as well

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tration of one in ten thousand, whereas the periosteal fibroblasts react optimally at a concentration of one in three million If periosteal fibroblasts are derived from another and younger embryo still another dosage is needed to obtain an optimal reaction with mitosis These results suggest that fibrocytic elements from different sources and from animals at different ages can have different sensitivities or can change their sensitivity toward growth hormone

WILLIER raised a question as to whether estrogen exerts a conditioning effect on the uterus preparatory to the action of some other hormone such as progesterone SEGAL answered that this has been worked out very carefully in the rabbit by McPhail (1934), who showed that progesterone has no proliferative effect on the endometrium unless it is preceded by a priming dose of estrogen BURNS cited the mammary gland as another example of this relationship The immature mammary gland of the rabbit will not respond to prolactin and secrete milk until it is first conditioned with estrogen Another example can be cited from the amphibians The gonads of *Rana catesbiana* a species which has a very long larval life lasting into the *second year* undergo sexual differentiation into ovary and testis rather early and then remain in a relatively inert condition for a long time, until metamorphosis or later Puckett (1940) showed that during this quiescent period of development it is impossible to affect the gonads by administration of sex hormones unless gonadotrophin is given at the same time If gonadotrophin is given the gonads are sensitized to sex hormones, and transformation of sex is obtained SCHNEIDERMAN gave still another example from insects The juvenile hormone has no action at all unless the prothoracic gland hormone ecdysone is present The juvenile hormone modulates the effects of ecdysone on the cells of the insect It apparently has no action of its own

TONUTTI agreed with Segal that the full action with progesterone is obtained only when there is pretreatment with estradiol But Hooker and Forbes (1947) found that there is a marked swelling of the nuclei of the uterine stroma without any preceding estradiol treatment SEGAL then commented on the different situations encountered in these two cases In the McPhail test where estradiol is required first for a priming purpose the condition of the entire uterus is evaluated, and the proliferation of the endometrium is examined histologically In this case estradiol is almost certainly effective in causing a vascular proliferation prior to the progesterone effect it thus appears that for progesterone to have an effect on the entire gland there must first be this preparation of the vascular bed and an increase in the vascularity of the uterus TONUTTI pointed out that in the McPhail test the epithelium of the en

dometrium becomes higher and larger under the influence of estradiol SEGAL agreed that this is so to a limited extent Glandular proliferation is minimal with estradiol alone, if there is any at all With the Hooker Forbes test where there is a direct action of progesterone alone, there is probably no real necessity for the vascular bed to be increased previ ously Progesterone is administered locally, directly to the cells The cells are exposed to the progesterone immediately without the necessity for an increased vascularity beforehand

WEISS again summarizing indicated that each gland should be con sidered as composed of a glandular constituent, a stromal constituent and a vascular constituent In the past, little attention has been paid to the specific reactive capacities of the blood vessels and connective tis sue they were considered to be the same over the entire body It now seems more likely that all three components of each gland are harmoni ously differentiated in the same way Thus all three components of an organ would seem to be specialized The other possibility is of course that the primary action of a hormone is exerted on any one component which then secondarily affects the other ones This type of situation is encountered in the gonads where the female component releases some thing that inhibits the male component The decisive experiment would be to break up an organ into its three components by pure culture and then treat them alike Will only one component respond or will all three respond? The former should be the case if estrogen for example exerts a primary effect on one component of the uterus But Weiss sus pects that it will be possible in such an experiment to demonstrate an independent reaction of each component to the hormone He asked whether tests of this sort had ever been made He strongly suspects that the mosaic properties of the vascular tree and of the connective tissue will prove to be just as striking as the mosaic properties of the epithelial organs with which they are associated

GAILLARD stated that he had not done quite the experiment suggested by Weiss but a related one If the ovaries of 3 week old mice are cul tured the typical stroma changes during cultivation and the typical in terstitial cells disappear If these cultures are then treated with gonado trophins there is no reaction whatsoever But if the organ cultures are grafted back to a littermate the typical interstitial elements reappear Thus the stroma has retained its original specificity which it did not manifest under in vitro conditions it then manifests its original speci ficity upon its return to the body Except for these observations of Gail lard no one seems to have made observations of the type suggested by Weiss Both WILLIER and WEISS emphasized that this general problem has been neglected by endocrinologists as a group

IX

Ontogeny of Selected Hormone-dependent Receptors Feather Papillae and Pigment Cells

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The topic was introduced by DR WILLIER who recalled that many excellent studies have been made on the feather papilla and its contained melanoblasts as endocrine receptors Male and female chickens of certain breeds can be distinguished from one another by the marked sexual dimorphism in the structure of their plumage If a genetic female is castrated the feather papilla produces in certain regions of the body, a feather which is typically male in its structure Since this is not true of all feather papillae there apparently exists a differential sensitivity to the female sex hormone in the population of feather papillae Each bird possesses approximately nine thousand such papillae, and each one gives its own distinctive and individual response to hormones Feather papillae of castrated males likewise construct a male type feather which differs from that of the normal male only in that it continues to grow almost indefinitely If a feather is plucked in a castrated male it begins to regenerate within 6 days If it is plucked from a normal male, it will not regenerate until the next molting period If the female hormone is added to either a castrated male or a castrated female the feather papillae produce female type feathers

Plumage may show sexual dimorphism in pigmentation as well as in structure One of the breeds in which melanoblasts are sensitive to sex hormone is the Brown Leghorn In the male the breast feather is uniformly black while in the female it is more or less salmon colored Complete castration of both male and female birds results in plumage that is male type in both structure and pigmentation The breast feathers are then uniformly black in both genetic males and genetic females in the total absence of sex hormones Male hormones are unable to cause a change in the pigmentation of such castrates Only an ovarian graft or an injection of estrogen can bring about a change in pigmentation from black to salmon the female coloration Under the influence of estrogen the melanoblasts of the growing breast feather differentiate into red melanophores rather than into black ones Thus the melanoblasts of a

regenerating breast feather of either sex react to female, but not to male hormone they are sensitive to estrogens but are insensitive to androgens (Willier, 1950, 1953) The hormonal milieu can clearly change the direction of differentiation of the melanoblast in the Brown Leghorn

Saddle feathers of castrates are long and acuminate Their coloration is red primarily, indicating the action of pigment cells which produce red pigment granules, although there is a small spot of black coloration indicating as well the action of pigment cells containing black pigment granules Addition of estrogen causes the formation in regenerating feathers of a peculiar pattern which is a curious mixture of black and red pigment granules the structure of the feather is also changed (see Willier 1950 Fig 2) Thyroxin also exerts an effect on plumage coloration in this breed in general it causes an extension of the amount of black pigment deposited within the feather during its development

In a few instances races have originated in which the males exhibit hen feathering almost identical with that of the females i e, there is little or no sexual dimorphism in plumage structure In the complete absence of sex hormones in castrates of either sex the feather papillae form cock feathers which are like those of normally cock feathered breeds in shape and structure In other words the feather papillae of these breeds are conditioned to respond to either sex hormone equally well they are insensitive to differences between them The Silver Campine is an example of such a hen feathered breed There is little or no sexual dimorphism in feather structure or pigmentation The adult plumage is barred black and white except for the hackle feathers, which are mostly white In normal adult bird no red pigment is visible in the definitive feathers yet a few red pigment cells may be present in regenerating feathers After castration of the male the plumage assumes the form of feathers characteristic of cocks of cock feathered breeds and red pigment appears in many regenerating feathers (Nickerson 1946) Thus in the absence of male sex hormones the formation of red pigment is no longer suppressed The administration of either testosterone propionate or estradiol again suppresses the formation of red pigment and in appropriate feathers the black and white barred pattern is restored (see Willier 1950 Fig 3) The melanoblast responds to each kind of sex hormone alike It is therefore insensitive to differences between estradiol and testosterone

Willier has summarized the evolution of the response of feather papillae and pigment cells to sex hormones (1950 Fig 1) The starting point for this scheme is the Jungle Fowl which exhibits sexual dimorphism in structure and color much like the Brown Leghorn In such breeds the

female hormone acts as the "sex differentiator" for the plumage A mutation produced the breeds with hen feathering, in which the feather papillae and pigment cells are sensitive to the presence of sex hormones but are unable to distinguish between male and female sex hormones Other breeds have originated which have pigment cells that are insensitive to sex hormones In such breeds the sex hormonal milieu can be changed repeatedly and even in a drastic manner, and still pigmentation is not affected Still other breeds have evolved lethal type pigment cells which are hormone insensitive Examples are the White Leghorns or White Wyandottes, which possess pigment cells, but these cells die prematurely before they have an opportunity to deposit pigment in regenerating feathers, consequently, the feathers remain white But, if pigment cells from such breeds are cultivated in vitro they form abundant pigment WILLIER then presented his interpretative scheme of the interaction of feather papilla, melanoblast and hormones encountered in the production of color patterns (Willier, 1952, Fig 3)

The nature of the feather papilla was then examined in more detail It is a complex morphologic unit which regularly regenerates a new feather after each molt or plucking throughout the life span of the bird It is composed of a specialized dermal papilla which during regeneration becomes encircled by a thickened ring or 'collar" of epidermal cells the collar is invaded by melanoblasts All the structural elements which make up the vane (rhachis and barbs) of the definitive feather originate as discrete ridgelike outgrowths or sprouts from the apical margin of the epidermal collar Melanoblasts enter these sprouts from the collar Each sprout constantly increases in length by the addition of cells at its base from the collar it also increases in length by elongation of the cells within the sprout The number of melanoblasts fed into each forming sprout is exactly controlled in some way as is their multiplication within the sprout There is a certain population density of these pigment cells that is precisely controlled

Intersecting the outgrowing barb ridges at a level just apical to the epidermal collar is a narrow transverse zone which is quite free of visible pigment cells However melanoblasts are present in this zone as has been shown by appropriate tests of isolates for their capacity to form melanophores At the apical border of the pigment free zone the melanoblasts first begin the synthesis of visible melanin pigment granules and soon assume the characteristics of typical branched melanophores It is here that the direction of differentiation of hormone sensitive melanoblasts is first influenced by sex hormones For example when female sex hormones are added to Brown Leghorn castrates the melanoblasts in

this region no longer differentiate into black melanophores but now differentiate into melanophores containing red pigment As long as female sex hormone is present in adequate amounts, red pigment will be produced and deposited at the base of an otherwise black feather If female hormone is not added, black melanophores then appear in this zone of differentiation Thus the period during which female hormone was present during the development of a feather is recorded permanently as a red band across the feather Thus it is possible by injecting female hormone and then by discontinuing injections and then by injecting them again, etc to produce a feather with alternate black and red transverse bands Such patterns were first produced by Dr Mary Juhn She did not go to the laboratory on Sunday and her failure to inject female sex hormone on that day was recorded permanently as a black bar on a red feather such bars could appropriately be called Sabbath bars

Other complex experiments have been performed on feather papillae by Wang (1943 1948) He removed dermal papillae from feather follicles in the saddle region and then transplanted them to feather follicles in the breast region which were deprived of their dermal papillae The epidermis of the empty breast follicles covers the transplanted dermal papillae originating from saddle follicles and the composite feather papillae then form feathers whose form is characteristic of that of the *breast* feather tract The transplanted dermal papilla from the saddle region exerts an inductor like action on the epidermis of the breast follicle but the response that is obtained is the only one that the breast epidermis is capable of giving namely formation of a typical breast type feather If female sex hormone is given to the castrated bird possessing such a composite feather papilla and treatment is then discontinued a transverse red bar can be recorded on this feather just as if it were developing from a normal breast feather papilla

Thus the feather papilla is a remarkable example of an endocrine end organ which can be rather thoroughly analyzed with respect to its powers of response to changes in the hormonal milieu And as stated before there are about nine thousand different feather papillae on one bird each responding in a different way to changes in the hormonal milieu Different papillae even within a single feather tract give different responses Thus the response of endocrine receptors can vary with their positions on the body

Burns then raised two questions He inquired first whether the lethal action encountered in pigment cells of White Leghorns and White Wyandottes which destroys them before they have an opportunity to deposit pigment is due to a mutation acting within the pigment cell or

whether it acts primarily in the surrounding tissue, thereby killing the pigment cell indirectly WILLIER answered that there is no question about this, it is acting within the pigment cell itself If White Leghorn pigment cells are transplanted to a black breed they cause formation of white feathers on that breed When they get into a feather papilla that normally would contain pigment, they still behave just as they would in a papilla of a White Leghorn They die precociously They form some pigment, and they also form pigment in vitro as has already been mentioned But they suddenly pull in their processes and die Occasionally they even deposit a few pigment granules before they die but only rarely Their life span in tissue culture is about 3 days contrasted with the life span of pigment cells of Barred Plymouth Rocks which survive *up to 10 days after explantation* This has been worked out carefully by Hamilton (1940) The gene for white in White Leghorns is a dominant gene Other white breeds such as White Plymouth Rocks, have a recessive gene for white, their pigment cells die even more precociously in vitro than those of dominant white breeds Thus it appears that there is no question that the lethal factor is operating within the pigment cell itself There is also another line of evidence in favor of this conclusion When pigment cells are transplanted from any colored breed to White Leghorn hosts, these pigment cells survive in feather papillae of the latter and produce and deposit their pigment thus forming in host feathers a color pattern typical of the donor

The second comment made by BURNS was more in the nature of a remark than a question He emphasized that the feather papilla is a receptor which in a particular part of the body, produces a feather of a particular morphology and particular shape which can be changed by the action of hormones And this papilla contains pigment cells which produce a pigment the color of which changes with certain types of hormones In all these experiments the changes have presumably been induced by injection of pure steroid hormones Have all these changes been induced systematically by transplantation of gonad tissue and if so, do the results correspond *exactly* with those produced by pure steroid hormones? He emphasized that this is an important question relative to the problem as to how closely pure hormones represent or approximate the natural hormone Apparently the feather germ provides a system in which this problem can be checked rather minutely to determine whether pure hormones do produce exactly the same changes with respect to the form of the feather with respect to the color of the pigment with respect to the band pattern etc as do grafts of the natural gland WILLIER answered that female sex hormones do produce exactly the same effects

turns black with approach of the breeding season but remains ivory colored all year round Hence a castrate during the breeding season has a bright colored nuptial plumage but the quiescent ivory colored bill This indicates that melanin deposition in the bill is controlled by gonadal hormones Injection of androgen into a castrate male or female results in blackening of the bill Injection of androgen into a female or a male out of breeding season (castrate or intact) fails to bring on melanin deposition in regenerating feathers After several attempts to induce nuptial plumage development with androgens and other steroid hormones had failed, it was observed that this result could be achieved by the administration of pituitary extract It should be emphasized that none of the non hypophyseal hormones—and all were tested—would give the response This aggregate of results indicates that a hypophyseal factor has a *direct* action in causing melanin deposition by the melanocytes of the breast feathers By the method of elimination to find the active principle it was discovered to be the luteinizing hormone (LH) of the anterior pituitary It has since been found that human chorionic gonadotrophin and pregnant mare serum, both containing the luteinizing principle also cause feather blackening in these birds This effect is directly on the melanocytes of feather tracts and is not mediated through the gonads, since a positive response is obtained in castrate birds

In a closely related bird the Paradise Wydah LH causes the deposition of melanin not only in the feathers but also in the bill Castration of the Paradise Wydah causes no change whatsoever in the seasonal dimorphism Both feather and bill blackening continue to occur annually in castrate males Injection of LH can induce the appearance of both nuptial characteristics—black bill and black breast feathers—in a female or in a male during the quiescent season

Two points deserve particular emphasis First in the same bird the Napoleon weaver finch the same cell type in different locations responds to different hormones The melanocytes of the bill are unaffected by LH directly but are stimulated to deposit melanin by androgen The melanocytes of the feathers are unaffected by androgens but are stimulated to deposit melanin by LH directly Second between the two types of birds, the same morphologic structure—the bill—has changed its responsiveness from one hormone to another In the case of the Napoleon weaver finch it responds to androgen, in the Paradise Wydah it responds to LH

WEISS inquired whether adrenalin is effective SEGAL answered that adrenalectomy of these birds has never been performed but that attempts have been made to elicit this coloration response with corticoids

or with adrenocorticotrophic hormones, without success TONUTTI expressed his surprise that a tissue can react to LH without intervention of the gonads He inquired about the kind of LH that was used SEGAL answered that this is one of the few examples of the response of a peripheral tissue to a trophic hormone, peripheral, that is, outside the gonads The LH that was used came from various sources The so called purified LH fraction was obtained from all the suppliers in this country like Armour Li in California provided some, as did McShan at Wisconsin and others who have been attempting to purify LH as such

SCHNEIDERMAN pointed out that this is one of the most striking examples of the ability of an end organ to capture hormones Certainly these two end organs seem to capture different hormones and to have changed the hormones that they were originally responsive to SEGAL agreed that this does appear to represent an evolutionary shift from the direct use of gonadotrophic hormone to perhaps a later stage of evolutionary history in which the intermediary action of the gonads comes into play

WEISS then inquired whether it is possible that some other organ is providing the hormone in certain cases of so called hormone insensitive pigment cells which show no change in behavior in the absence of a particular gonad Perhaps two glands share the same function and, if so, the removal of both glands would be necessary to demonstrate that the pigment cells are actually hormone-sensitive Perhaps the action of thyroxin in extending pigmentation of the feathers is a tendency in that direction MUHLBOCK inquired whether Willier had done any experiments involving antagonism between the male and female hormone He mentioned that he had carried out such an experiment some years ago (Muhlbock 1939) but that no antagonism was indicated In his experiments, testosterone did not counteract the effects of estrogenic hormone He suggested that someone with Willier s experience might learn more about the mechanisms involved with the use of such an approach

X

Synergistic Action of Hormones

Weiss stated that two agents are said to be "synergistic" whose effects are additive along any particular linear scale involving for example, size of the nucleus or cell, thickness of an epithelium, number of cells etc If two agents produce subtractive effects along the linear scale they would be exerting inhibitory or antagonistic actions It is not possible to analyze the real operation of a synergistic system but only the external criteria, which provide the only way of measuring the system, and they are limited Burns mentioned the possibility of confusing what has already been discussed as a conditioning effect of one hormone followed by the action of another with true synergism which implies two hormones acting together simultaneously When two hormones are administered separately in time, one definitely in advance of the other, a true conditioning effect might be suspected but it is also possible that when two hormones are given simultaneously, one hormone is conditioning while the other is exploiting what has been conditioned Weiss agreed that the fact that two hormones are administered simultaneously does not guarantee that the cell will be exposed to both of them at the same time or in the same way It is true that all too often the time of administration is confused with the time of action about which literally nothing is known There is always a lag period which varies depending on permeability and accessibility, and this ought to be emphasized

Willier recalled again, as he had done earlier in the introduction to the conference that Smith (1933) was the first to emphasize that a synergism occurs between thyroxin and a growth promoting factor from the anterior pituitary He showed that an anterior pituitary extract, not a purified hormone has a growth promoting effect on hypophysectomized rats and that administration of thyroxin simultaneously causes an augmentation of growth beyond that produced by the pituitary extract alone Subsequently Evans and Simpson and others have shown with purified hormones that the so-called growth hormone has a promoting effect on growth which becomes even greater when thyroxin is given simultaneously

The work of one of his collaborators Dr de Voogd v/d Straaten (unpublished) was then discussed by GAILLARD He has been studying the effect of estrogen and of progesterone either separately or in combination on the process of fibrocytosis in human neutrophilic granulocytes The particles to be phagocytosed are composed of shellac, Janus green, and normal actylic alcohol and are attached to the fibrin fibers of the clot on which the granulocytes are distributed at random The granulocytes move along the fibers and phagocytose the particles In the presence of 0 075 μg of estrone, about 70 per cent of the granulocytes derived from a male appear to have ingested one or more particles Progesterone (0 05 μg) leads to roughly 65 per cent of the cells with phagocytosed particles Addition of 0 075 μg of estrone plus 0 05 μg of progesterone to the same preparation leads to about 90 per cent of the cells showing phagocytic activity In control preparations without any hormone added to the medium about 59 per cent of the granulocytes show phagocytosis The high percentage of cells exhibiting phagocytosis after the simultaneous addition of the two hormones suggests a synergistic effect WEISS pointed out that the effect of the two acting simultaneously is actually more than the sum of the effects of the two hormones separately Thus the two hormones mutually reinforce each other in some way But, since they work in the same direction this certainly is a case of synergism GAILLARD then emphasized that the above results are obtained only with exactly calibrated dosages No effect at all occurs with the wrong doses

Reactions of leukocytes from females are different and are related to the menstrual cycle Granulocytes taken from the pre ovulatory period do not react after the addition of estrogen Phagocytosis increases significantly however, if a combination of progesterone and estrogen is given Granulocytes taken immediately after ovulation behave quite differently The addition of 0 1 μg of estrogen gives a significant rise in the percentage of cells showing phagocytosis while the combination of progesterone and estrogen produces a decrease in the number of cells containing phagocytosed particles From the twenty fourth day of the cycle on this effect becomes less pronounced and by the time of the menstrual bleeding the effect characteristic of the pre ovulatory period reappears It seems that the observed differences in behavior depend on the amount of estrogen and/or progesterone present in the female blood

SCHNEIDERMAN asked whether the blood cells were washed and put in the serum GAILLARD said this was not done They used 0 05 cc of capillary blood to which was added 0 02 cc of the reagent containing the gum acacia boric acid and particles to be phagocytosed as well as

the hormones to be tested The mixture is introduced into a capillary chamber and is centrifuged slightly during ¾ minute before coagulation occurs In this way a red and a white clot are obtained The cells in the white clot are used for the observations after an incubation period of 2½ hours at 37° C Dr de Voogd v/d Straaten has studied seven complete cycles, and with the exception of one case the effects were as described above

WEISS pointed out that there appears to be an actual sex difference in the leukocytes GAILLARD answered that the activity of the leukocytes of the female is different from that of the male when they are in female serum But if the male leukocytes are given the same combination of estrone and progesterone, they behave just as if they are female WEISS then asked whether this means that there is no basic sex difference in the leukocytes of males and females Is their behavior due entirely to the serum containing them? GAILLARD stated that he did not believe this to be true SCHNEIDERMAN then inquired what happens if the cells are washed GAILLARD answered that washing probably induces considerable change in the surface of the cells although they have never actually studied this WEISS pointed out that this is another striking example of the effects of sex hormones on the properties of a cellular population that would not normally be suspected

TONUTTI then discussed an observation of Hisaw *et al* (1954) which is pertinent to the topic Two estrogens—namely, estradiol and its metabolite estrone—both have a growth promoting action on the uterus But if these two estrogens are given together their effects are not additive Estrone seems to have an inhibiting action on the effect of estradiol This shows how complicated the interaction of two hormones can be even when they have the same specific effect WEISS asked whether this is necessarily conclusive If a detector is used which has a ceiling on its production can you step up that production above its ceiling? Obviously, once a reacting tissue has reached its maximum capacity it cannot be driven any higher He wanted to be certain that this possibility is excluded TONUTTI answered that they use a very low dosage of estradiol If a higher dosage is used it is very easy to increase the weight of the uterus five to ten times This then avoids the possible criticism suggested by Weiss

SEGAL mentioned that they (Wicks and Segal 1956) have just finished repeating this work in mice and that some additional comments are pertinent The inhibiting interaction of the two estrogens can be summarized as follows The uterine response to estradiol in immature female mice is inhibited significantly by concurrent injection of estriol

The extent of inhibition is proportionate to the amount of estriol added The impeding interaction occurs along the ascending segment of the estradiol dose response curve and with higher doses as well In no case does a combination of these estrogens have a positive additive effect These conclusions are based on both wet and dry uterine weight determinations Day by day analysis shows that uterine weight gain during each 24 hour period of estradiol treatment is inhibited when estriol is given concurrently The total results negate the possibilities that the estriol estradiol interaction is a masked summation effect in which time of maximum effect has shifted or that an additive effect occurs in which the two estrogens combined exceed the maximum response dose Rather it appears that estriol competes actively at a physiologic site essential in the development of the usual estradiol stimulation of the uterus WEISS inquired whether this could be tested by changing the timing of administration of the two with respect to each other SEGAL stated that this is to be the next step in the program WEISS asked whether these two molecules are closely related in structure SEGAL replied that they are very similar WEISS then suggested that the results might be explainable by the population density of the two types or that there might be an actual steric interference at the same site just as in the case of structural analogs But he also pointed out that the discussion is now centered around inadequate replicas of some natural agents and that their effect might not apply in the natural situation

SEGAL then commented on the way Szego and Roberts (1948) would interpret an experiment of this sort They believe that, before estradiol can exert an effect on a target organ it must first be conjugated with a protein probably at the liver site Estriol is the more polar of these two compounds and by virtue of this character it would have a tendency to enter into a protein complex more selectively Thus perhaps the site for activation of the estrogen are being selectively occupied by the estriol by virtue of its greater polarity

MOOG then commented on the work of Bullough (1950) on mitosis in vitro He took small pieces of ear epidermis and studied its rate of mitosis in tissue culture The rate of mitosis is always rather uniform in epidermis taken from males provided that the tissue is taken at the same time each day By contrast the rate of mitosis differs considerably in epidermis taken from females depending on the time of the cycle when the epidermis is taken If it is isolated at a time when a high titer of estrogen is expected in the blood stream of the female the isolated material continues to show the influence of estrogen for a num

ber of hours Apparently, the epidermal material carries along a supply of the hormone which lasts for some time But if the epidermis is kept in isolation for several hours, the estrogen effect disappears The mitotic rate then drops

WEISS then inquired whether there is any synergistic effect that works on mitosis by way of the pancreas There are two agents which can produce mitotic stimulation locally when grafted under starved amphibian skin One is the central nervous system, which has a special *carbohydrate requirement the other is the pancreas They seem to have* nothing in common except that carbohydrate metabolism is important to both He also inquired about the possibility of an inner antagonism between insulin and glucagon, the products of beta and alpha cells, respectively TONUTTI stated that it is well known that steroid diabetes can be induced in the guinea pig with high doses of cortisone (Hausberger and Ramsay, 1953, 1955) Fabbrini (1955*b* 1956) studied in his laboratory the influence of cortisone on the nuclear size of the alpha and beta cells in the guinea pig Only the beta cells show an increased nuclear volume The alpha cells remain unchanged The ratio of alpha to beta cells (normally about 20/80) remains unchanged Since the islets become very large there must be an increase in both beta and alpha cells Mitosis of beta cells also occurs When the animals are *treated simultaneously with cortisone and insulin the beta cells remain* unchanged, their nuclear volume and the number of mitoses do not increase under these circumstances

WEISS then asked whether cortisone alone causes a mitotic response of the skin He inquired about the response to cortisone in wound healing TONUTTI stated that it is diminished in the rat but not in the guinea pig WEISS then inquired whether the reduction in wound healing is in terms of mitotic activity or of migration of cells GAILLARD answered that it is the migration of cells which is affected SCHOTTE *recalled that Manner (1955) had recently demonstrated a decrease in* mitotic activity in the skin of the newt after application of cortisone

Attention was then called by WILLIER to the studies of his student Konigsberg (1954) and others on the level of blood glucose in the chick embryo The concentration of blood glucose is expressed as milligrams per cent Presumably glucose is present in the blood from the onset of circulation It enters the blood from the yolk sac, which according to Claude Bernard, functions as a liver The blood sugar level rises until the tenth day of incubation remains relatively constant until the fourteenth day and then increases considerably by the sixteenth day (see Konigsberg, 1954 Fig 1) What regulates these changing levels

of blood glucose? What causes these shifts in steady state with time? By 10 days the thyroid follicles are beginning to accumulate thyroglobulin Even earlier, adrenalin or noradrenalin is present in the embryonic adrenals Ascorbic acid and lipids are accumulating in the adrenal cortex during this period and these products are indicators of the functional activity of the cortex The beta cells appear in the pancreas by the twelfth or thirteenth day All these endocrine glands are producing hormones which are involved in sugar metabolism in posthatching stages at which times their actions are interlocked in complex ways Whether the hormones produced by these embryonic glands are actually released into the circulation is uncertain but in mammals capillaries become associated with the beta cells of the pancreas at about the time that the secretion granules appear in these cells, providing the necessary structural relationship for release of insulin into the blood The anterior pituitary gland is certainly involved in some way, directly or indirectly because there is a marked elevation in the blood sugar level following hypophysectomy (Konigsberg 1954, Fig 1) Much remains to be learned about the development of homeostasis or steady states in the embryo, and the factors which control the blood sugar level are especially intriguing because of the shifts in steady state of this level with time

SCHNEIDERMAN asked whether it is possible that the regulation of the level of blood sugar is simply the consequence of changes in the kinds of tissues that are present at different stages Perhaps there is no real hormonal regulation at all in these early stages but simply a series of changes in the distribution of glucose between the new tissues MOOG stated that this is not very likely because in the very period when the leveling off of blood sugar occurs there is a loss of glycogen from the liver It would seem that the blood sugar level should increase under those circumstances WILLIER asked Moog if she has confirmed the plateau period in any of her work She replied that she has not done any blood sugar work but that she has worked with liver glycogen and does find a definite drop in the latter at the time that the level of the blood sugar remains constant BURNS mentioned that Jost (1953) reported a big drop in liver glycogen in the rabbit fetus after the pituitary is removed by decapitation Seven to 8 days after decapitation the *liver glycogen dropped to about 15 per cent of its original value*

WEISS stated that he felt these remarks to be particularly significant when the work of Landauer and Zwilling is recalled (Landauer 1945, 1947*a* *b* Landauer and Bliss 1946 Landauer and Lang 1946 Zwilling 1948) They demonstrated the significance of maintaining an appropriate steady state because the slightest deviation from this state has

very serious morphogenetic effects on development If insulin is injected around 4 days, hypoglycemia results, and the embryos that develop are rumpless, sirenoids, or exhibit various other kinds of malformations of the skeletal system Apparently the level of blood glucose at each stage of normal development is harmonized with the requirements of that particular stage When the level falls below the requirements, developmental abnormalities are the consequence Weiss inquired what happens if insulin is injected as late as 12 days of incubation He wondered whether it has an effect after the homeostatic mechanism is established The question remained unanswered Weiss also inquired whether there is an effect of the insulin on the pancreas when it is injected later Does it cause a compensatory retrenchment of pancreatic differentiation? Again there was no information

WILLIER pursued the matter of the complexity of the endocrine control of sugar metabolism even further, pointing out that it is necessary when development of these systems is studied, to keep in mind what is known about the adult The adrenals produce adrenalin which exerts a hyperglycemic effect It can act directly on the liver It can also act through the hypothalamus then through the posterior pituitary via neurosecretory nerves then through the portal circulation between the posterior pituitary and anterior pituitary and the anterior pituitary can send a message to the alpha cells Embryologists must explain how these things become established during the formation of the embryo and determine the time at which they come into play When are the e mechanisms established? Are they established in a certain sequence? MOOG inquired whether the thyroid should not be invoked also, in view of the work of Konigsberg (1954) WILLIER agreed that the thyroid certainly plays a role Moreover, there is evidence that the so-called purified growth hormone has a hyperglycemic effect the idea being that it acts on the alpha cells This has been worked out by Young (1953) in England

TONUTTI mentioned that in his laboratory the influence of the so called growth hormone on the ratio of alpha to beta cells in young rats was studied by Thiermer (1953) and that this ratio remained unchanged The rats respond well with accelerated growth following administration of growth hormone preparations but there is no detectable influence on the alpha cells This study does not substantiate Young s claims. At least in the morphologic sense no action of the growth hormone on alpha cells is demonstrated This was an Armour growth hormone that was used WILLIER then asked whether Tonutti would agree that the growth hormone has a hyperglycemic effect TONUTTI

agreed that it has a diabetogenic action but he emphasized that this does not necessarily mean that the diabetogenic action is exerted through the alpha cells

MUHLBOCK then turned the discussion to some of their work at the Amsterdam Cancer Institute where the center of interest is the relation ship between hormones and the genesis of cancer, especially in mice The main object of study is the genesis of mammary cancer conse quently interest centers mostly on the function of the hypophysis and ovaries It is well known that the hormonal function of the ovaries can be followed readily by taking vaginal smears The ovarian function in various strains of mice has been followed throughout life by means of vaginal smear studies and a curious phenomenon was observed The normal estrous cycle lasts for 4–6 days and cycles normally follow one another quite regularly Frequently however this regular periodicity is interrupted by prolonged 8 day diestrous intervals Van der Lee and Boot (1955 1956) investigated the meaning of these diestrous periods They demonstrated that these periods represent spontaneous pseudo pregnancies This means that all hormonal changes occur as in a normal pregnancy except that there are no fertilized ova and hence the placenta is absent This period of pseudo pregnancy lasts only half the time of a normal pregnancy that is 10–12 days When fertilized ova are trans ferred from another animal into the uterus of a pseudo-pregnant mouse the ova undergo implantation and the pseudo pregnancy is converted into a true pregnancy in which the young are born at term (Boot and Muhlbock 1953)

Such spontaneous pseudo pregnancies are observed regularly only when at least two female mice are kept together in a cage Female mice kept in isolation show the phenomenon much less frequently When a number of mice that had been kept separately for a period of time are subsequently put together four to a cage nearly all the mice become pseudo pregnant at once It is of course known that pseudo pregnancy can be induced by mating with a sterilized male mouse Consequently it was suspected that perhaps these spontaneous pseudo pregnancies were induced by close bodily contact among the females But it was then found that if the mice are kept four to a cage but separated from each other by a partition of wire mesh spontaneous pseudo pregnancies continue to occur The role of sight and smell in this phenomenon was then tested Ablation of the olfactory lobes in the $C_{57}B1$ strain is fol lowed by nearly total disappearance of spontaneous pseudo pregnancies the sequence of the cycles then becomes quite regular (Fig 10) Thus an olfactory stimulus appears to be responsible for the induction of

these pseudo pregnancies This means that an olfactory stimulus can lead to the release of prolactin from the anterior lobe of the pituitary WEISS asked whether the results are any different if females are exposed to males rather than to other females MUHLBOCK replied that this experiment has not been done

WILLIER remarked that *such experiments reminded him of the results* obtained by Olsen and Marsden (1954), of the Agricultural Experiment Station at Beltsville, Maryland Turkeys occasionally lay parthenogenetic eggs They observed that a greater percentage of parthenogenetic eggs is obtained from those females that can see a gobbler in the distance

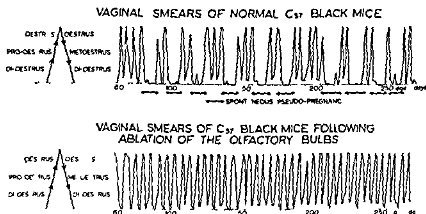


FIG 10 —Effect of ablation of the olfactory bulbs upon the occurrence of spontaneous pseudo pregnancies (Mühlbock original)

WEISS then discussed some experiments carried out with Dr Jane Overton (Weiss and Overton 1954 Overton 1955) If amphibian urodele larvae are starved, the mitotic activity in the skin is reduced *from sixty dividing cells per thousand cells examined to practically zero* The drop in mitosis is rather sudden If a graft of nerve tissue is placed under the skin mitotic activity flares up locally in the starved animal and then fades out again If the animal is first starved and then is well fed mitotic activity goes up and comes down to about 50 per cent of its maximum even when feeding continues This response occurs over the entire skin, so there is enormous mitotic activity at this time WEISS suspected that part of this stimulation might be the result of the presence of food, the rest due to actual consumption of food Observations by Overton (unpublished) seem to indicate that this might be the case If the animals are kept completely starved and are then shown food

but are not permitted to eat it, they show a mitotic wave in the skin WEISS suggested that this works from the visual centers through the hypothalamus to the pituitary and from there perhaps through the pancreas Apparently there are quite a number of similar situations in which the nervous system acts through the hormonal system to affect growth KOLLROS inquired whether the sense of smell might be involved rather than vision WEISS answered that he did not know the experiments in detail

A remark by Professor Pumphrey (1955) was then read by WILLIER Pumphrey is a neurologist but he was invited to make the introductory remarks at the conference on comparative endocrinology of vertebrates held at the University of Liverpool in 1954 'It was almost exactly twenty years ago that C N Long then in Philadelphia, coined the epigram 'the pituitary is the conductor of the glandular orchestra This observation is still quoted and still seems apt but it has I think acquired a *new look* Today if I have the picture correctly the pituitary conducts by permission and each member of the orchestra has a clause in his contract entitling him to switch the conductor off when he has had enough The concept of feedback which has overrun neurology is obviously casting its long shadow over endocrinology WILLIER then added paraphrasing Pumphrey to some extent that it seems surprising that questions of feedback were not raised twenty years ago The fact that these questions are being asked today is a measure of the change in emphasis which has taken place in twenty years This feedback mechanism is a most extraordinary one and has been neglected The pituitary stimulates an endocrine receptor the endocrine receptor produces hormones which are poured into the circulation When a certain quantitative level is reached they somehow or other tell the pituitary to stop

WEISS then commented that feedback mechanisms also act between homologous organs and that this problem has been analyzed mathematically (Weiss and Kavanau 1957) Briefly each organ or each cell type produces inhibitors of its own growth and the total concentration of these in the circulation comes into equilibrium with the productive or generative mass to produce a steady state Any interference with the products in circulation will automatically shift the equilibrium away from the intracellular concentration of inhibitors in the direction of the pool so that part of the cellular content will be 'disinhibited so to speak automatically This works for liver cells and kidney cells and for several specific glandular types Dr Teir (Teir *et al* 1957) is working this out for the supraorbital glands It also works for the different types

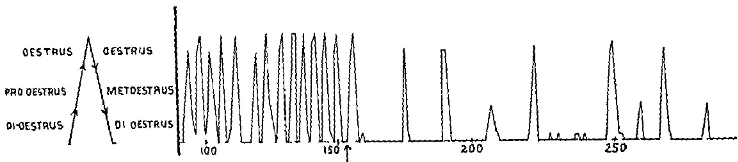


Fig 11 —Estrous cycle in mice before and after subcutaneous implantation of 5 hypophyses of the same strain ↑ = time of implantation Age is indicated in days Redrawn from Mühlbock and Boot (1957)

of blood cells during blood regeneration It is quite possible that a similar mechanism operates between any pair of endocrine organs so that what one releases acts as an inhibitor of the other Instead of the cell releasing autoinhibitors it acts on the paired or matched organ the product of which will serve as an inhibitor for that particular organ Thus the pituitary for example would inhibit itself through the thyroid instead of through its own agency Perhaps the hormonal mechanisms of inhibition can be visualized as the evolution of specialization of the more general feedback system

KOLLROS then called attention to an experiment of Blount (1935) in which a salamander larva is made hyperpituitary by grafting to it two extra pituitaries at the rudiment stage Growth and metamorphosis are then studied Metamorphosis does not occur early but takes place at the same time in normal controls and in the triple pituitary animals Thus some regulation occurs to control the production of TSH at normal levels but apparently the production of growth hormone is not controlled since the body proportions of operated animals are substantially changed although the over all size of the triple pituitary animals is substantially less than that of controls

MUHLBOCK stated that he has done experiments in which extra pituitaries are transplanted subcutaneously in mice (Muhlbock and Boot 1956) By examination of the estrous cycle in the operated animals it can be demonstrated that frequent pseudo pregnancies occur (Fig 11) It follows that the hypophysis when transplanted subcutaneously produces prolactin independently of the hypothalamus

XI

Integration of the Endocrine System

Emphasis was placed by Dr Weiss on the development of the endocrine *system* How does harmony develop between the endocrines and their receptor organs? And by "receptor organs" is also meant other endocrines which are at one time an emitter and at another time a receptor Actually, it is doubtful whether there is any functional cell in the body which does not produce some effects on other cells via the circulation The only difference between the endocrine system proper and the production of other types of cell specific secretions that may affect other types of cells may be the fact that the endocrine system works with predestined types of receivers Thus in the endocrine system there are pairs of matched organs an emitter with an attuned receptor Non endocrine systems may work in a more general way But on the molecular level it may be that something produced by the skin may have a very specific molecular receptor elsewhere Even within the endocrine system the statement that matching pairs of organs are involved is an oversimplification which has only been introduced into the literature because some of the paired responses are the most conspicuous ones Practically every one of the endocrine glands has some less conspicuous effects which are of a more universal nature affecting metabolic processes all over the body There is a vast humoral communication system in which each tissue can communicate with all the other tissues by mechanisms with different degrees of chemical specificity Chemical specificity means that there is a coded message that operates by virtue of something like the steric conformity of a molecule like the way a coded message works when punched on an IBM card That code has all degrees of detail or specificity, but it is a very universal language which affects all cells to a certain extent as in the case of those that affect metabolism in general or calcium metabolism or sugar metabolism More organs in the future may have to be included as part of the endocrine system

Tonutti then discussed the whole interacting endocrine system as a unit rather than the individual pieces of the system He pointed out how difficult a task it is to outline in a schematic manner the interrelations

among the different endocrine glands It is possible to give a picture of the basic actions and interactions of hormones only in an oversimplified manner (Fig 12)

It is well established today that the hypothalamus exerts control over many functions of the hypophysis such as production and release of ACTH, TSH FSH, and LH (see Harris 1955 Fields, Guillemin, and Carton, 1956) As was pointed out by Dr Muhlbock earlier the production of luteotrophin (LTH) is independent of hypothalamic control, since this hormone is also produced by pituitary grafts placed under the skin in heterotopic positions STH or the so called growth hormone seems to have some indirect control over the islands of Langerhans and the parathyroids but there are some intermediate mechanisms which are not understood at the present time The indirect influence of STH on the islands of Langerhans is mediated by the blood glucose (*A*) and the influence on the parathyroids by the level of blood phosphorus (*B*) (see Tornblom 1949, Engfeldt 1950) Thyrotrophic hormone (TSH) influences the structure of the thyroid and stimulates production and release of thyroxin and triiodothyronine (*C*) Both products of the thyroid diminish the production and release of thyrotrophin from the anterior pituitary by a feedback mechanism (*D*) Both hormones can also diminish the growth promoting action of a given amount of TSH on thyroid cells (*E*) This interference of thyroid hormones with the action of thyrotrophin on thyroid cells can be demonstrated in the hypopysectomized rat The growth promoting effect of a given amount of TSH (as revealed by measurement of the height of thyroid cells) is diminished if the animals are treated simultaneously with thyroxin (Rawson and Money 1949) or with triiodothyronine (Fabbrini 1955*a*)

ACTH influences the structure of the adrenal cortex and stimulates the production and release of cortical hormones (*F*) Only glucocorticoids have a pronounced influence on the production and release of ACTH from the pituitary (*G*) FSH acts on testis tubules and stimulates spermatogenesis (*H*) LH (or ICSH) promotes the differentiation of the Leydig cells of the testis (*I*) or ovaries (*J*) and stimulates these cells to produce androgens and estrogens Both androgens (*K*) and estrogens (*L*) are able to inhibit the gonadotrophic activity of the anterior pituitary The structure and function of testis tubules are also under the influence of androgens produced by the Leydig cells under the control of ICSH (*M*) It is well known that tubular damage without disturbance of testicular androgen production may stimulate the production and release of gonadotrophins McCullagh *et al* (1950) believe therefore that the tubules produce a second testicular hormone called

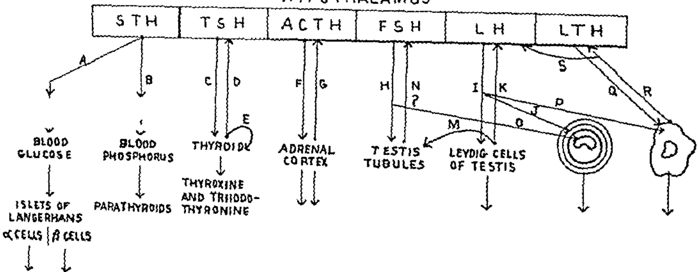


FIG 12 —Interactions within the endocrine system (Tonutti original)

inhibine" which counteracts especially the FSH activity of the pitui tary (N) The existence of this hormone is doubted by other authors who suspect that it is simply estrogen produced by the tubules or by the Leydig cells

FSH is responsible for the growth of secondary follicles and the formation of mature follicles (*O*) But even in the complete absence of the pituitary secondary follicles of small size are still growing The production of small follicles is therefore an autonomous process, and only the formation of medium sized and mature follicles is dependent upon the presence of FSH Theca interna tissue is produced simultaneously with the growth of follicles but there is no production of estrogen unless stimulated by LH (*J*) In the absence of this hormone as a consequence of hypophysectomy the cells of the theca interna and all cells which are derived from the theca interna (interstitial cells of the ovary or ' theca organs) undergo atrophy On the other hand, administration of LH to hypophysectomized animals leads to pronounced hypertrophy of the theca cells and interstitial cells, and the estrogenic activity of the ovary reappears

Weiss inquired how much of the increase in diameter of the follicle is true growth He pointed out that in the case of the eye much of the increase in diameter is due to the production of vitreous humor To what extent does LH stimulate true growth i e increase in number and size of cells and to what extent does it merely stimulate production of follicular fluid? Quite different mechanisms are involved in the two instances and it is essential that each effect be analyzed separately Burns then referred to some experiments on growth of ovarian follicles in the axolotl after hypophysectomy (Burns and Buyse 1932) The animals were hypophysectomized as embryos The ovaries developed quite well and the egg cells entered the ovocyte stages and grew until they obtained a diameter of approximately 400 μ At that stage the yolk granules are first laid down in the egg Exactly at that stage all growth of egg cells ceases in hypophysectomized animals Similarly if adults are hypophysectomized when the ovary is full of mature eggs all follicles containing mature eggs degenerate All follicles containing yolk even in the smallest quantities degenerate But those follicles which have not yet entered the stage of yolk formation persist in an apparently unchanged condition In other words the adult ovary after hypophysectomy reverts exactly to the stage of differentiation which the embryonic ovary is able to attain without the hypophysis Something associated with the phenomenon of yolk metabolism is apparently involved in the arrest that occurs

TONUTTI stated that the growth of follicles has been analyzed in his laboratory by Dr Fetzer In hypophysectomized rats, secondary follicles remain with a diameter of 150 μ The nuclear size of the granulosa cells of these follicles is the same as in follicles of the same size in normal animals When the follicles grow in the normal animal the nuclear size first diminishes After maturation of the follicle, the nuclear size of the granulosa cells increases and reaches its maximum at the time the granulosa cell is transformed into a lutein cell after rupture of the follicle

After this diversion, Tonutti returned to the discussion of the interactions shown diagrammatically in Figure 12 After rupture of the mature follicle the granulosa cells are transformed into the granulosa lutein cells under the influence of LH (*P*) But the cells of the corpus luteum do not secrete progesterone until they are stimulated by the third gonadotrophin namely LTH (*Q*) Progesterone then acts back on the production of LTH (*R*) and LH (*S*) by the pituitary

Gonadotrophins are not secreted in appreciable amounts before puberty There remains the question concerning the factors which induce the pituitary to begin secretion of gonadotrophins at the time of puberty Recall the case of the Leydig cell tumor in the five and a half year old boy discussed earlier Under the influence of the androgenic activity of the Leydig cell tumor this boy reached a bone age of approximately twelve years In this patient there was a detectable excretion of gonadotrophins in the urine and true Leydig cells were present in both testes Thus the precocious pseudo-puberty induced by androgens was already transforming into a true but incomplete precocious puberty It appears that the pituitary begins to produce gonadotrophins automatically when the body has reached a certain degree of somatic maturation and not at a certain chronological age

Just the opposite situation seems to be demonstrated by another patient sixteen years of age (121 cm) who lacked sexual development This boy with secondary hypogonadism and dwarfism was treated for 3 years with chorionic gonadotrophin (LH activity) Within 3 years complete sexual maturation occurred and the patient increased 20 cm in height and showed somatic maturation When treatment was stopped after 3 years he continued to grow and gonadotrophins were excreted into the urine The following interpretation seems indicated The androgens produced by the Leydig cells under the influence of chorionic gonadotrophin induced somatic maturation After a certain degree of maturation was reached corresponding to the degree of maturation at the time of onset of puberty in a normal individual the hypophysis began to secrete gonadotrophin

These two contrasting cases demonstrate that there exists some prin ciple of harmony between somatic body maturation and the gonado trophic activity of the pituitary In the former case the tumor with its androgen production brought the boy to a bone age of twelve years, the whole body underwent maturation, and thus STH and all the other fac tors were produced by the hypophysis In the second example the six teen year old boy was brought to a bone age of fourteen to fifteen years by treatment with chorionic gonadotrophin When this bone age was reached, the boy s own hypophysis began to produce FSH and also STH and its other secretions The same effect would probably have been pro duced, had testosterone been given The treatment seems only to induce the maturation of the body, subsequently and as a consequence of this, the pituitary begins normal and physiologic production of hormones Androgens are anabolic hormones (growth promoting factors) and so are STH and gonadotrophic hormones

WEISS pointed out that the hormones produced by the gonads nor mally check the production of gonadotrophic hormones by the pituitary Yet in the present situation, gonad hormones appear to stimulate pitui tary activity TONUTTI answered that the action of the hormones pro duced by the gonads depends upon the concentration used Androgens decrease LH production only when they are present in very high con centrations WEISS also pointed out that in the second example there is a priming of a self perpetuating chain reaction whereas in the former case a course of events is started which leads to stabilization at a lower level The mechanisms involved seem obscure but the examples clearly demonstrate some degree of interaction between the two parts of the system namely degree of maturation of the body and onset of produc tion of hormones by the pituitary gland These are certainly not sep arate compartments of the body which develop independently of one another

TONUTTI then gave an additional example He stated that patients with secondary hypogonadism generally have a somewhat lowered basal metabolism i e a diminished function of the thyroid If they are treated with gonadotrophins or with androgens this defect in metabolism is cor rected without introducing thyroid hormone WEISS inquired whether corticoid androgens exert an effect on pituitary activity MOOG agreed that there certainly exists the possibility of abnormal androgen produc tion by the adrenal cortex and stated that the fetal cortex normally pro duces androgens BURNS pointed out that while the suggestion by Weiss would be true in the case of the adrenogenital syndrome there is no evidence that such a relationship exists in the normal individual MOOG

insisted that it certainly exists in the fetus Price inquired whether testicular androgen stimulates the germinal epithelium of the seminiferous tubules Moog stated that there is no good evidence of this in normal individuals

Weiss then inquired whether there should be indicated on the diagram a direct interrelation between parathyroids and estrogen, at least in the case of birds, in which estrogen is involved in calcium metabolism in certain types of bone Tonutti stated that he did not believe this relationship between parathyroids and ovaries to be a direct one Gaillard stated that, as far as he knew, it is not certain that estrogen interferes with the production of hormone by the parathyroids Rather it seems to interfere with the reaction of the end organ It interferes in some way with the metabolism of the end organ, which prevents its proper response to the parathyroid hormone The end organ in this case would be the fibroblastic series of cell types connected with the bone matrix osteoblasts, osteoclasts and morphologically non differentiated fibroblastic cells Perhaps it would include the organic mucoproteins of the matrix as well Gaillard also suggested that the interrelationships between total calcium total protein, and calcium ions as demonstrated by McLean and Hastings (1935) should be kept in mind in any consideration of the regulation of the activities of the parathyroids

Martinovitch inquired whether the production of STH and LH is really absolutely independent of control by the hypothalamus Weiss answered that if removal or addition of STH or LH changes other glands which are under hypothalamic control, then these hormones form part of the total system Willier mentioned that there is some evidence to indicate that adrenalin acts on the anterior pituitary whether it acts through the hypothalamus is not quite certain He stated that he did not think Tonutti meant to imply that all actions on the diagram are directly through the hypothalamus or that the hypothalamus must act through the neural lobe in order to get to the anterior pituitary He recalled the experiments of Courrier (1951) who showed by labeling the iodine atom of thyroxin that this hormone when administered is concentrated selectively within the neural lobe and not within the anterior pituitary Thyroxin thus seems to act back on the pituitary via the neural lobe Weiss asked whether the posterior pituitary is merely a storage organ or whether it is a conversion point where certain things happen Or is it a combination of the two? Tonutti answered that he thought it acts *in a way similar to the thyroid which splits the hormone away* from thyroglobulin Willier added that what happens in the neural lobe as far as the mechanism is concerned is unknown Tonutti

stressed the fact that the capacity to form hormones is a specific quality of the pituitary itself The hypothalamus only modulates the rate of production of hormones by the anterior pituitary

WEISS then stressed the lack of discrimination in general between the effects of hormones on growth, on differentiation, on production of se cretion and on discharge of secretion In each case the action of hormones should be described in at least these four parameters A fifth effect as GAILLARD pointed out would be on the transformation of secretion drop lets before transfer, i e on conversion The secreted droplet is not nec essarily the same as the one which is discharged The cell of a gland like the thyroid first secretes thyroglobulin Then an enzyme acts on the thyroglobulin, which leads to the formation of a product which can es cape from the gland and influence the organism WEISS pointed out that it can then influence the very cell from which it came (see Fig 1)

PRICE inquired whether the statement about production of secretion should be refined to include qualitative, as well as quantitative factors FSH causes the formation of an antrum and the production of follicular fluid but estrogen is not produced unless LH is present Thus the pro duction of secretion is not only quantitative it is also qualitative in this case WEISS agreed but pointed out that the type of secretion produced is determined by differentiation of the cell or by modulation Perhaps the cell can assume two different developmental forms thus producing two different products He insisted that the effects of hormones should be specified in terms of all five parameters before deciding which one is the source of the feedback mechanism Does the secretion produced by a cell act on that cell causing it to grow? Is there such a feedback mecha nism right inside the cell between the product of the cell and further growth of that cell? Or is it only after the product has escaped from the cell that it acts back on the source? Does it control production directly and perhaps through production the further growth of the cell? These and other questions were referred back to an earlier discussion involv ing Figure 1 TONUTTI suggested that there is considerable variation from one type of cell to another For instance if estrogen is given in low doses for a long time the result is diminished output of LH by the pituitary On the other hand if a large dose of estrogen is given for a short time there is a heavy discharge of stored LH from the pituitary of the rat This is the so-called Hohlweg effect (Hohlweg 1934)

WEISS summarized the discussion by stating that any outline such as that given in Figure 12 simply provides a blueprint for the future Each part of such an outline has to be examined in its own right Any all inclusive formula will probably not be found

XII
Mode of Action of Hormones

The attention of the conference was next directed to an analysis of *the way in which hormones act preferably on the molecular level How* do they act on structures, on enzyme systems, on metabolic processes, etc ? It is to be anticipated that an understanding of the mechanism of action of one hormone will not necessarily provide a key to understanding the action of any or all the others WEISS recalled the flush of excitement a few years ago when it was thought that the mechanism of action of insulin was known It was thought then that all other work on hormone action could be dropped because *the* mode of hormone action had been discovered Nobody is so naive any longer In the case of insulin the steroids and thyroxin there is at least some information concerning the action of the hormone on the cell and its special equipment

KOLLROS called attention to the work of one of his students Stephanie Barch (1953) He recalled that Etkin (1934) working on the metabolism of metamorphosing tadpoles stated that there was no change in oxygen uptake on a dry weight basis But Wills (1936) working in Witschi s laboratory found during the pre metamorphic and metamorphic period an indication of oxygen uptake increase at this time Mrs Barch worked on skin over the stages when the skin and other tissues of the tadpole are obviously being stimulated by thyroid hormone but at a time when the skin has not yet responded structurally by obvious physical changes During this interval she found an increase in oxygen uptake of isolated skin on a dry weight basis She also obtained this response in tadpoles whose skins were stimulated by local implants containing thyroxin WEISS pointed out that this is another example of the fact that by the time changes can be seen with the microscope the interesting events are long since over He wanted to know how thyroxin produces this change in metabolism How does the hormone relaxin act on mucopolysaccharides? How do hormones act on enzyme systems? On mitochondrial populations? What is known about the actual point of attack of any of these hormones on the molecular level?

MOOG stated that there is no real evidence of any direct action of

hormones on enzyme systems either in vivo or in vitro The best evidence that has been presented recently suggests that, whenever there is a change in enzymes due to hormone action, the effect is probably due to the action of the hormone on the synthesis of the enzyme protein rather than on the enzyme directly WILLIER said that he supposed that hormones accelerate enzymatic action by perhaps acting as coenzymes WEISS suggested that the hormone might also produce a structural change in the cell which would permit the existing enzyme population to operate more efficiently per unit number of enzyme molecules Of course, the actual enzyme efficiency depends on the probabilities of collision between the enzyme and the substrate An ordered arrangement of the enzymes, if it is promoted by structural provisions in the cell leads to the type of effect that Kollros had just described SCHNEIDERMAN pointed out that in insects attention has been paid for a long time to changes in enzyme systems after hormonal treatment but that more recently attention has been focused on changes occurring in the intracellular structure after hormone administration Recently Wigglesworth (unpublished) has shown that within a few hours after injection of the bloodsucking bug *Rhodnius* with the prothoracic gland hormone ecdysone the entire intracellular structure of the epidermal cells is changed The endoplasmic reticulum looks very different the mitochondria change in shape etc These events occur exceedingly rapidly and are paralleled by other changes—for example by increased respiration which is presumably a result of changes in mitochondria and increased protein synthesis which may be the result of changes in the endoplasmic reticulum The suggestion has been made that ecdysone and possibly other hormones act by affecting intracellular permeability Consider for a moment how a cell might regulate its metabolism Certainly one way is to keep enzymes away from their substrates One way to start reactions going is to bring enzymes into contact with their substrates Thus some hormones may act by facilitating or restricting the access of enzymes to their substrates Such mechanisms may be very important in the regulation of metabolism WEISS agreed that it is possible that the primary change is in cellular organization and that any change in enzymatic activity is a secondary derivative of a more direct primary change SCHNEIDERMAN added that this might be a change in mitochondrial permeability

WEISS then inquired about the actual penetration of hormones into cells as against surface action Do they operate at the surface and does this engender a change which propagates itself into the cell? Or do they actually enter the cell? Do certain hormones enter and others work on

the outside? Perhaps the proteins behave differently from the steroids which have lipid receptors in the cell surface

Willier emphasized that an important aspect of the problem is how large molecules get into the circulation from the endocrine glands and once in the circulation how these large molecules are picked up by receptor organs He emphasized also that they can leave and enter the circulation only through the walls of the capillaries, and he stressed therefore that the capillary wall may play an important role in the transfer of large molecules Weiss however, stated that it is not necessary to stress the role of capillary walls because hormones exert their actions in vitro when endocrine glands and receptors are cultured together Capillaries add a complication only in vivo He inquired whether Willier intended to imply that there is an actual screening of molecules at the capillary bed, which either retains the hormone in the circulation or allows it to enter the cells If so this cannot be the general situation because of the situation in vitro The problem remains as already stated Does the hormone merely affect the surface of the cell and, as a consequence of this action does the cell then proceed on its own? Or does the hormone actually enter? Willier stated that his working hypothesis was that, once the molecule gets into the capillary wall is selectively picked up by it the molecule is then bound to the cell membrane by some counterpart in the latter which it matches Once this combination occurs the cell membrane becomes active somehow or other in transforming the molecule into something simpler which enables it to act within the enzyme substrate system within the cell This in turn results in the synthesis of a different product within the cell

Schneiderman thought that studies have been made in which labeled thyroxin is injected into an animal and subsequently the liver is homogenized and fractionated, to find out in what particular fraction the thyroxin is found Segal objected that this gives information only about the location of the iodine I^{131} may be lost rapidly from the thyroxin molecule and may well enter cells independently of the rest of the molecule Schneiderman stated that this necessitates the use of N^{15} labeled thyroxin or something else This would enable the investigator to find out the particular cell organelle in which the hormone is concentrated

Weiss then inquired whether Dr Price's enzyme studies with the Manns on the seminal vesicles give any indication of how the hormone works in mobilizing fructose Price answered that Mann (1954) had reviewed the evidence that testicular hormone normally is essential for the production of fructose by accessory glands He outlined a series of enzyme reactions which might be involved in the conversion of blood

glucose to seminal fructose and postulated that alkaline phosphatases may facilitate fructose liberation from phosphofructose Williams Ashman and Banks (1954) found ketose reductase in fructose producing glands and suggested that this enzyme might play a role in the conversion of glucose to fructose with sorbitol as an intermediary Hers (1957) has now confirmed this and has shown the second enzyme step in the transformation But the way in which testicular androgen influences these enzyme systems in the accessory glands is not known

MOOG then mentioned the work of Levine and Goldstein (1955) on the action of insulin on permeability These authors eviscerated rats and then injected them with insulin and also with sugars which are not utilized by the muscles and other cells of the body In this way, by separating the penetration from the utilization of sugars they were able to demonstrate that insulin facilitates the penetration of these sugars just as much as it facilitates the penetration of glucose, which is utilized

WEISS pointed out that there may be a selective receptor mechanism in the cell surface placed there for recognition or rejection of a hormone molecule Once accepted perhaps this molecule can be passed down the line by the bucket brigade of active transport Once inside the cell it may have an entirely different activity The mechanism of reception and the mechanism of action may even be connected with different parts of the molecule The molecule may have a tag which is essential for its recognition and reception and it may have another part which is instrumental to its proper function inside the cell The part of the molecule that works inside the cell by promoting intracellular permeability may be different from the prosthetic group that causes the molecule to be accepted MOOG pointed out that this certainty might be expected to be true in the case of proteins WEISS suggested that perhaps the structural analogs of the steroids have a different spectrum of action largely due to differential acceptance rather than to differential mechanisms of action once they enter

SCHNEIDERMAN inquired whether it is necessary to think of selective pickup of the hormone by the receptor organ Is it not possible that it just happens that particular cells can use the hormone whereas other cells which also get it cannot use it? WEISS answered that the labeling experiments demonstrate that the hormone is concentrated by a particular gland and therefore it is selectively picked up SCHNEIDERMAN agreed that this is certainly true of some hormones but that it cannot be assumed a priori that this is the way that all hormones behave

WEISS concluded that nothing much is known about the real mode of action of any hormones He inquired whether anything is known about

how the hormones act in the production of cancer in response to steroid hormones MUHLBOCK replied that practically nothing is known on the cellular level WEISS then asked about the action of hormones on the ground substances rather than on the cells How does relaxin affect the mucopolysaccharides? GAILLARD replied that it affects them by acting on calcium exchange WEISS then inquired whether this action occurs through the connective tissue cells or whether it is a direct attack on the ground substance GAILLARD stated that it is generally assumed that its action is directly on the ground substance He mentioned that gradients of action on the ground substance spreading from cells are not seen The fact that has been observed is that the staining intensity of the ground substances is increased It is assumed that the reaction is more intensive because smaller molecules are produced by depolymerization WEISS asked whether the only studies of the reaction are those based on the Schiff reaction He inquired whether some straight biochemical studies have been made But, even then, only the product would be known and not the process behind the product

WILLIER listed three hyotheses about hormone action based on a review of the literature (1) the hormone acts by bringing about changes in concentration of enzymes in the tissues, (2) the hormone functions as a component of the enzyme system, as a coenzyme (3) hormones act by having a direct or indirect effect on the acceleration and/or inhibition of enzyme systems For example in a recent study in vitro it was found that a particular homogenate could carry on the normal processes of synthesis of a hormone independently of the trophic hormone but that, when the latter was added these processes were accelerated SCHNEIDERMAN again remarked that another way that hormones may act is by changing intracellular permeability, thereby enabling an enzyme to come into contact with its substrate WILLIER questioned whether this should be called permeability ' but WEISS reminded him that what is called permeability nowadays is becoming somewhat hazy SCHNEIDERMAN continued by stating that inside the cells things are kept away from each other there is compartmentation One of the obvious way for nature to affect cellular activity is to allow compartments to mix It is conceivable that this is one of the ways in which hormones can act WEISS agreed and stated that this is what he implied in speaking of structural facilities for the reaction but he stated that he had difficulty in visualizing how this could result in switching cellular activities into alternative courses SCHNEIDERMAN replied that one compartment is affected in one case another in another etc

WEISS suggested that this is only passing the buck from the cell as a

whole to the cellular compartments SCHNEIDERMAN defended his re marks by stating that the analysis is brought down at least one addi tional step to the intracellular level WEISS replied that, even so, it is not brought down to the molecular level How do hormones act to cause the cell to switch into a different line of differentiation? He called at tention to the experiments of Fell and Mellanby (1953), in which the development of skin cells can be switched by treatment with vitamin A into a course which will produce slime and cilia This is a model of events in the vagina under the cyclic action of steroid hormones The two agents may act through a common point of attack He pointed out that in his own laboratory it has been demonstrated that it takes only 15 minutes exposure to vitamin A for the manifestation 5 days later of the switch in developmental fate (Weiss and James 1954 1955) The fate of the whole protoplasmic system has been changed rather dramat ically by a very short exposure which is definitely, as far as indirect evidence is concerned confined to the surface It does not penetrate Perhaps these experiments provide a model of hormone action GAILLARD inquired whether the concentrations used in these experiments are not exceedingly high in comparison with the concentrations of hormones WEISS replied that they are not high in his experiments The concentra tion is 1/10 000 vitamin A in 5 per cent alcohol for 15 minutes There after the cells are completely washed The cells that show the effect later are not the same cells that were treated but are about the third genera tion progeny Thus the level of vitamin A falls below that characteristic of normal plasma

SCHNEIDERMAN suggested that the enzyme systems that have been studied intensively are mostly those concerned with mobilizing energy These have proved exceedingly uninteresting as far as hormone action is concerned The reason that analysis of hormone action has not progressed effectively on the molecular level is that hormones affect many synthetic reactions and in such cases the enzymes involved are un known WEISS agreed and reminded the group that statements exist in the literature of embryology to the effect that specific enzymes or the reactions catalyzed by them control such processes as determination morphogenesis or differentiation So far this has not been proven for any enzyme (Boell 1955 p 548)

SCHNEIDERMAN then suggested that perhaps before going immedi ately from the response of the entire cell to the molecular mechanisms involved some idea concerning what happens to the ultrastructure of cells shortly after hormone treatment should be obtained by the use of the electron microscope WEISS answered that he has been doing this for

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sized as he had done earlier, that there is a great difference from one organ to another When thyrotrophic hormone is incubated with fresh tissue it is inactivated but it is not used up in the usual sense of the term WEISS answered that at least it is not being recirculated, it is lost as far as additional activity is concerned Is this an accurate model of what happens in the body? SCHNEIDERMAN stated that if a trophic hormone is to be able to regulate some gland it just cannot sit around for a long time It has to be there to exert its effect, and, if its effect is to be stopped it has to be broken down WEISS suggested that the hormone as a whole may not be the catalyst The part of the molecule that acts as a catalyst may not be used up but it may then be unrecognizable because it has lost its specific tag of recognition, once this part of the molecule has been released from the total hormone molecule

MOOG pointed out that steroid hormones are metabolized very rapidly SEGAL stated that they may be metabolized in the liver rather than at their site of action WEISS emphasized that if they are destroyed in the liver they must first come out of their site of action and this would provide indirect evidence that they are only passing through receptor organs KOLLROS stated that the ones which are destroyed in the liver may simply be the ones which failed to reach the receptors GAILLARD mentioned that ACTH is extremely labile in pure plasma Within 24 hours after it is added to plasma its activity is literally absent because of disintegration of the active substance

BURNS emphasized that the case may be very different not only for different end organs but for different types of hormones Exactly opposite to the case described by Tonutti where a part of a receptor does not grow to any larger size after the rest of it has been removed although it is exposed to the full concentration of the hormone is the situation in which compensatory hypertrophy of one ovary occurs after removal of the other one This has always been explained on the basis of the fact that with one ovary gone the other somehow or other profits by the excess gonadotrophin which is in circulation WEISS pointed out that O M Robertson (unpublished) had demonstrated the same response for one testis of a trout following removal of the other one MARTINOVITCH mentioned that the adrenal responds similarly But WEISS then emphasized that Robertson s trout were said to have also responded when hypophysectomized KOLLROS then mentioned that this is the old pattern of the crab claw in regeneration WEISS noted that he had quoted a large amount of literature of this kind in his article on specificity and growth control (Weiss, 1955) Such reactions are scattered throughout the entire animal kingdom This is actually what led to his hypothesis

several years and that useful information has resulted but that such an approach is leading down only one order of magnitude which is still far removed from the molecular level The point to emphasize is that almost nothing is known about the mode of action of the hormone at its terminal point, by which its action is gauged How the hormone furnishes a signal to which the receptor can respond is not known One of the great absurdities of modern research is the discrepancy between the amount of work that is being done at the end of purification, crystallization, and identification of hormones versus the shortage of work on the action of hormones Perhaps it is because the latter task is so difficult or because no one sees a way to attack it

MARTINOVITCH inquired whether the hormone activates the substrate, i e whether it acts as an enzyme He pointed out that Tonutti feels that hormones are not used up in the chain of reactions following their administration WEISS stated that this raises the question as to how much of the hormone can be recovered after it has acted TONUTTI mentioned that Selye (1949) reduced the size of a receptor organ to see whether the response of the reduced receptor would be greater to a given amount of hormone than in the normal situation He found it to be no greater Naturally such an experiment would be valid only if all the administered hormone were accumulated by the receptor organ If the hormone is distributed equally over the entire body reduction in size of a small receptor by half would have no significance WEISS felt that this approach is too indirect to be useful He inquired whether anyone has ever extirpated a thyroid from a fully stimulated animal transplanted it to a hypophysectomized animal, and demonstrated the escape of residual thyrotrophic hormone from the graft in an amount sufficient to stimulate host thyroids Assuming that the thyroid does concentrate thyrotrophic hormone and not degrade it can it be gotten out of the thyroid again by grinding up that thyroid and putting it into a hypophysectomized animal to see whether there is at least a temporary flaring up of host thyroid activity?

GAILLARD reviewed an experiment of his which is somewhat like the experiment suggested by Weiss He added parathormone to a culture medium in a given concentration and allowed it to act on bone tissue which underwent resorption as a consequence of this hormone action He then took some of the fluid in which this action had occurred and placed it in a new container along with another piece of bone which in turn underwent resorption WEISS pointed out that this merely means that hormone is still present in the medium it does not mean that it has or has not, entered the receptor apparatus TONUTTI again empha

the hypophysis produces no luteotrophic hormone Corpora lutea are formed at ovulation but they do not produce progesterone

WEISS inquired whether the number of eggs produced by the two ovaries is approximately the same MUHLBOCK answered in the affirmative He said that they make a routine practice of transferring fertilized eggs and practically always find the same number BURNS recalled that in the original experiments on unilateral ovariectomy mentioned earlier the remaining ovary after removal of the other one always matures approximately twice the normal number of follicles for that ovary MUHLBOCK stressed the importance of the use of pure bred strains for experiments of this type GAILLARD suggested that the site of implantation is of importance as well as the strain If the ovary of the mouse is transplanted together with its extra-coelomic cavity, a successful graft is obtained whereas if the ovary is grafted alone and no free surface is provided the graft does not take MUHLBOCK disagreed and stated that in their experiments the results were the same in either case BURNS said that he knew nothing about the situation in the adult but that in the case of the embryonic ovary of the rat, a small free space is essential around it in order to obtain survival and good differentiation just as Gaillard had described

GAILLARD mentioned that when suprarenal glands are grafted into the brain they take but do not function until the moment the animals are adrenalectomized

KOLLROS then discussed the field of amphibian metamorphosis in relation to the problem of the mode of attack of hormones It is obvious that before the thyroid acts the pituitary must act by producing thyrotrophic hormone What it is that induces the pituitary to increase its production of thyrotrophic hormone at a particular time and thus to bring about the precipitous events of metamorphosis is not known Etkin (1936 1939) demonstrated by transplanting the thyroid gland adjacent to the pituitary gland that the pituitary produces thyrotrophic hormone a great deal earlier than the climactic events of metamorphosis indicate A very much accelerated metamorphosis results It is presumed that thyrotrophic hormone from the pituitary gland reaches the adjacent thyroid gland by diffusion thereby activating it The histologic response of the thyroid gland confirms this Presumably the amount of TSH released by the pituitary at the early stages studied is insufficient when diluted in the circulation to have an effect upon the thyroid gland at its usual site There is no evidence to indicate how much earlier the pituitary might be producing and releasing TSH

Questions have been raised concerning the onset of reactivity of the

of a steady state for the total volume of any organ system, irrespective of how it is divided into pairs of organs, scattered islets, or what not *It is the total mass that is under control* In the case of the testes of the trout, there would be a direct action between the testis on the left and the one on the right through the humoral system of the body If one is removed, the other enlarges In addition to such a mechanism acting *according to the principles developed by* Weiss and Kavanau *(1957)*, there is apparently a second system of growth control involving the pituitary and the production of gonadotrophins WEISS suggested that in all endocrine glands both mechanisms are probably demonstrable He *raised a question about the old Halsted law of transplantation* which states that if a supernumerary organ is added by transplantation it is better to get rid of the counterpart first and thus to reduce the total mass of that particular organ system He inquired whether this also *holds true for the endocrine system* GAILLARD *answered in the affirmative*, except for the anterior pituitary MARTINOVITCH stated that the anterior pituitary can be transplanted in any amount and will take As many as ten have been transplanted successfully

MUHLBOCK stated that after unilateral ovariectomy in mice the remaining ovary shows compensatory hypertrophy (Muhlbock and Boot 1956) As a measure of the function of the remaining ovary the number of ova released at ovulation is counted On the average, 5 ova per ovary are found in the normal animal After unilateral ovariectomy the remaining ovary produces double that number If an additional ovary is implanted subcutaneously either at the time of unilateral ovariectomy or some weeks later, compensatory hypertrophy of the ovary *in situ* is not inhibited If it is postulated that the hypophysis releases only a limited amount of gonadotrophic hormone, it may be concluded that this hormone is effective only on the ovary *in situ* The gonadotrophic hormone is effective only on the subcutaneous ovarian graft after extirpation of the remaining ovary *in situ* WEISS stated that a similar kind of observation has been made recently in fish by Peters in Tubingen (1955, personal communication)

WEISS then inquired about what happens immediately after hypophysectomy MUHLBOCK replied that in the rat with ovaries *in situ* a continuous state of pseudo pregnancy is found after removal of the pituitary gland from the sella to the kidney (Everett 1954 1956) This means that after its transplantation to another site than the sella i e away from the hypothalamus the hypophysis produces predominantly luteotrophic hormone During the estrous cycle of the normal animal

phically limited the other is not In the frog both of them depend upon metamorphosis

Thus in amphibian larvae certain tissues are responsive to thyroid hormone, while others are not Those which are responsive exhibit either progressive development such as the hind limbs beyond a certain stage or regressive development, as in the case of the tail the beaks and in the salamanders the tail fin, if not the entire tail Some of the structures which show progressive development do so because of the direct action of the thyroid hormone (Kollros and Kaltenbach, 1952 Kaltenbach 1953*a* *b* Kollros and McMurray 1956) Other changes are clearly the results of indirect hormonal action Helff (1928), for example demonstrated that the modification of the skin over the tympanic membrane depends upon influences emanating from the annular tympanic cartilage whose own prior changes are under the control of thyroid hormone

How can it be demonstrated that some tissue is directly responsive to hormone? This can be ascertained by transplantation of small pellets which contain thyroxin The first work of this kind was that of Hartwig (1940) He soaked pellets of agar in strong solutions of thyroxin and then implanted the pellets in the tail fins of salamanders The response consisted of a regression of the fin an indentation of the fin margin at the level of the implant Pellets of agar alone do not produce this result Kollros (1943) implanted thyroxin soaked agar pellets into the hindbrain of the frog tadpole The result was a precocious development of the hindbrain center which is responsible for the corneal reflex This is a local effect on a behavioral feature The performance of the reflex thus occurred in larval stages a great deal earlier than it would have normally Since the period of larval development is divided into an extensive series of well marked stages (Taylor and Kollros, 1946) the extent of this temporary disharmony can be indicated very clearly Thyroxin agar pellets liberate their hormone very quickly so that local effects are frequently masked by generalized effects resulting from the rapid accumulation of thyroxin in the blood Well compacted pellets of cholesterol and thyroxin combined in a ratio of 4 to 1 are better for this purpose since such a pellet if about 0 5 mm in diameter and less than 0 2 mm thick liberates its hormone over a period of a week or longer Although generalized hormonal stimulation is not avoided it is much less exaggerated than with agar base pellets and the striking local effects are generally easily distinguishable from the modest generalized ones (Kollros and Kaltenbach, 1952)

There has been a serious controversy, mainly between certain Ameri

tissues to the thyroid hormone It is known that in embryonic stages the tissues are not reactive However, some studies by Moser (1950) indicate that a whole series of tissues in the tadpole show a response at about the time feeding begins This takes place in the *Rana pipiens* tadpole at about a length of 10 cm A particular cell type in the brain which eventually responds to thyroid hormone does not become reactive until the tadpole is about 30 mm long (Kollros and McMurray, 1956) Thus different tissues become responsive at somewhat different levels of development WEISS inquired how this responsiveness is tested KOLLROS answered that resorption of the tail is studied by measurements on the shortening tail WEISS asked whether this begins at the 10 mm stage KOLLROS answered that it does if a great deal of thyroid hormone is given If thyroid hormone is added in substantial concentration to the medium in which the tadpole is swimming, starting at the time the tadpole is 8 mm long, i e, while it is still embryonic and utilizing its yolk, a shortening of the tail can be detected about the time it gets to be 10 or 11 mm This is a specific reaction to thyroxin, just as is stimulation of the hind limbs The hind limbs cannot be stimulated until after the limb buds have developed to a certain point WEISS mentioned that he had less confidence in the reduction of something as a criterion for hormone activity than in a positive criterion KOLLROS mentioned that Moser also used the loss of the beak as a criterion of response This again is a specific reaction occurring at no other time than at metamorphosis WEISS inquired whether jawbones form also or whether the reaction is simply the loss of something KOLLROS stated that Moser did not carry his experiments far enough to know He found that all four or five of his test structures showed the onset of tissue reactivity to thyroxin at about the same time The embryo is apparently unreactive to thyroxin until the 8 mm stage, and subsequently, in a given sequence certain tissues become reactive to the hormones

There are special differences between urodeles and anurans Certain reactions that are considered to be metamorphic in the frog are not metamorphic in salamanders The corneal reflex is one of these, since it develops in the hypophysectomized and thus non metamorphosing salamander, whereas in the frog it depends completely upon thyroid hormone and the metamorphic changes in the eye and brain (Kollros, 1942) Likewise, the pupillary response first appears at metamorphosis (Stone, 1930) Thus behavior may be listed as a functional index of hormone activity, and changes in behavior are involved in the whole complex of changes called collectively 'metamorphosis' Thus in the eye of the salamander one behavioral response appears to be metamor

pletely inhibit metamorphosis If larvae at the room temperatures of summer respond slightly to a given concentration of thyroxin, they may fail to respond at 17 C Or, if they still respond at 17°, they may no longer do so at 15° Thus speaking of hypophysectomized tadpoles treated only with external sources of d l thyroxin in the dose range of 0 01 to 1 0 μg/liter, the following elements which affect metamorphosis must be kept in mind (*a*) time of onset of tissue reactivity to thyroxin, (*b*) different thresholds for response by different tissues (remembering further that some responding tissues at first have high thresholds and later lower ones), (*c*) different rates of response of different tissues at any given effective hormone concentration, (*d*) influence of temperature levels upon either *b* or *c* or both

The studies of Kollros and McMurray (1955 1956) serve to illustrate points *a* and *b* The mesencephalic V nucleus cells in *Rana pipiens* apparently cannot respond to high concentrations of thyroxin by growth of both nucleus and cell body prior to stage III (length of about 30 mm) They respond slightly in stages III–IV and more completely at stage V, though they are probably not yet fully responsive even then (see Kollros and McMurray 1956 pp 3–6 and Table 1) When metamorphosis is stimulated in hypophysectomized animals with thyroxin concentrations barely adequate to bring the larvae to the stage of fore limb emergence (stage XX) or slightly beyond, many tissues change (skin glands develop molting occurs, legs develop fully, the operculum perforates, the extrinsic ocular muscles enlarge the gape of the jaws may increase), but such hormone concentrations appear to be inadequate to stimulate growth of the cells of the mesencephalic V nucleus (see Kollros and McMurray, 1956 pp 14 15 and Fig 6) Thus these unique easily recognizable cells of the optic tectum can be seen to be readily stimulated to enlarge by high concentrations of hormone at stage V but if metamorphosis is timulated over a long period of time by low concentrations of hormone the cells remain small, even though they begin to enlarge in the stage XIV–XVI interval Their failure to enlarge by the time stage XX is reached clearly implies that they are stimulated only by hormone concentrations substantially higher than those required to bring about the other metamorphic changes enumerated They are highly reactive cells but only with relatively high concentrations of thyroxin

One additional feature which might have been considered as item *e* above is the rate at which release of thyroid hormone occurs in the normally metamorphosing animal There is no assay for it prior to stage VII Apparently it increases very slowly at first as indicated by the

can and European investigators, concerning an interpretation of metamorphic mechanisms Moser (1950), for example, apparently assumes that particular metamorphic events require particular threshold levels of thyroid hormone to be reached or exceeded before they occur By contrast, Etkin (1955) asserts that any concentration of thyroid hormone adequate to bring about any of the metamorphic reactions is adequate to bring about all of them, given enough time for the action There is now sufficient evidence to rule against the latter idea in favor of the former i e, in favor of the idea of several specific thresholds Geigy (1941) reported no concentrations below one part in two billion and stated that this is ineffective Allen (1938) likewise stated that this concentration is ineffective Their negative results apparently stemmed from their use of normal tadpoles, in which the intrinsic hormone production and release mask the modest influence of thyroid hormone in low concentration In his own recent experiments the highest concentration used was one part in one billion (1 μg/liter) in the culture water This level is sufficient, for hypophysectomized and thus effectively thyroidless larvae for all events of metamorphosis through development of the legs perforation of the operculum and emergence of the forelegs, development of skin glands, and substantial loss or resorption of oral fringes, tail fin and axial structures of the tail With concentrations of thyroxin only 1 per cent as strong as this, certain metamorphic events can still be stimulated The development of the hind limb, for example, seems for a time to be independent of the thyroid gland since, in hypophysectomized tadpoles, it proceeds to the level of the normal stage VI–VII tadpole Then development terminates until stimulated by the introduction of hormones Stage VII is the stage at which one digit of the foot is separated from the common primordium of the other digits Treatment with low concentrations of thyroxin will stimulate further differentiation of the leg to the stage of two three four, or five toes Which of these stages is reached depends mainly upon two things the concentration of added thyroxin and the temperature If the concentration is correct to bring about the development of three digits further treatment at the same hormone concentration will bring about no additional change, even though such animals may be treated for an additional 12 months However, if the temperature is elevated by several degrees or if the hormone concentration is increased slightly during this time, some additional metamorphic progress is achieved i e four or five toes may be produced Temperature involves either the sensitivity of the responding tissues or their ability to respond at all (Kollros, 1956) It has been known for a long time that cold temperatures com

WEISS emphasized the value of the metamorphic process as a tool for the analysis of the action of thyroid hormone on the tissues There is one large cell on each side of the hindbrain of all amphibians and fish, called 'Mauthner s cell' This is one huge brain cell There is only one on each side of the animal characterized by its enormous nucleolus, large nucleus, and very large cell body This cell is surrounded by other large cells, the ones that Kollros mentioned As metamorphosis proceeds, this cell regresses its regression has been studied in *Xenopus* by Stefanelli (1950) After metamorphosis, it can no longer be recognized so its ultimate fate is unknown Weiss and Rossetti (1951) studied this cell under a variety of experimental conditions and also by implantation of thyroid directly into the ventricle against the area containing Mauthner s cell, so that the latter could be exposed to the direct influence of thyroxin from a localized source The implantation of fresh thyroid stimulates an enormous increase in mitotic activity in the ependyma of that area, neither boiled thyroid nor any other control gland exerts this action How this effect is brought about is unknown The volume of all the nuclei of the large cells in this area increases within a few days By contrast Mauthner s cell regresses One cell thus emancipates itself in its response from all the rest of the population and reacts entirely differently from the rest of them This certainly pinpoints selectivity even within a population of nerve cells! If this type of reaction is followed up in such a way as to determine how this cell differs metabolically structurally etc from its neighbors some clue concerning the point of attack of thyroxin may be provided

Thyroxin also has a striking action on muscle Recall that muscle consists of sarcomeres and that there are isotropic parts (I) and anisotropic parts (A) According to present electronmicrography actin fibrils run clear through the muscle fiber whereas myosin fibrils are short stubby pieces found only in the anisotropic zones associated with and probably wrapped around the actin fibrils At metamorphosis in urodele larvae most of the muscles remain entirely intact but the gill muscles disappear when thyroxin appears The I bands of the gill muscles are completely hydrolyzed and dissolved out, while the A bands float around as loose plates of myosin filaments which finally become secondarily dissolved Here again there is a very neat chemical fractionation of tissue components by thyroxin Another striking effect is on the basement lamella of the skin of amphibian larvae This membrane separates the connective tissue from the epidermis which in urodeles contains large glandular cells the Leydig cells and smaller basal cells and cover cells During metamorphosis this basement membrane is perforated

progress of limb development after stage VII Its concentration then appears to increase more rapidly, and even precipitously, at or just prior to metamorphic climax There is then evidence that the concentration declines, but just how far is not evident Studies by Barch (1953) have yielded interesting information on the rate of oxygen utilization by skin at different stages and on the responsiveness of the skin to thyroxin The first index of increase in thyroid hormone is the differentiation and growth of the legs, which though highly sensitive to the hormone at first respond to it very slowly Skin glands next develop The thinning of the skin window and shortening of the tail are both relatively in sensitive indicators of hormone concentration but, once adequate con centration of hormone has been reached, they respond rapidly In hypophysectomized animals, however, it is possible to achieve a very delicate balance of hormone concentration such that thinning of the skin window does occur but rupture does not If dosage levels are exactly right, the spiracle disappears and regression of the skin window area by erosion from the edge of the spiracle may go on being re stricted to the left side of the body By similar control of dosage, re gression of the tail may be stretched out over a period of several months rather than over some 6 10 days (Kollros unpublished)

The feedback mechanism remains to be discussed i e the influence of the thyroid hormone upon the pituitary gland There is a substantial reduction in the thyroid hormone content of the blood after metamor phic climax Is this reduction controlled by inhibition of TSH output by the pituitary? There is no evidence of such an effect during the long buildup period terminating after forelimb emergence Is the pituitary *in fact responsive to thyroid hormone at this time? It is responsive to* rather large doses of the hormone If a large thyroxin cholesterol pellet is implanted in a normal tadpole, a rapid spurt of metamorphic devel opment is recorded, beginning 2 or 3 days after implantation If the pellet size is gauged correctly, there will be too little hormone to bring about the climactic phase of metamorphosis and after a few days of rapid change the rate of change will decrease and may come to a com plete halt The tadpole may then remain unchanged for 3 6 or 9 weeks even for as long as 10 months (rarely) This block to metamorphosis is interpreted as thyroxin induced inhibition of TSH output, the re covery from which may be rapid or very slow Once metamorphic changes begin again the course of metamorphosis proceeds normally The metamorphic stasis indicates that the pituitary gland is sensitive to thyroid hormone but that ordinarily inhibitive concentrations of the hormone are not achieved prior to metamorphic climax

to forelimb emergence WEISS mentioned that in the normal metamor phosis of the European salamander, *Salamandra maculosa* this zone is very sharply defined as it moves up on the tail With the aid of the electron miscroscope it is possible to distinguish one cell on one side of the line that has metamorphosed and another cell on the opposite side which has remained larval in type SCHNEIDERMAN inquired concerning the fates of the larval cells KOLLROS and WEISS answered that they are resorbed

KOLLROS then added a few comments concerning Mauthner's cell A thyroxin cholesterol pellet was placed lateral to the medulla oblon gata just anterior to the level of this special cell Although the distance for diffusion of the hormone is considerable, there was probably less diffusion into the blood stream than in the experiments of Weiss and Rossetti (1951) Pesetsky and Kollros (1956) presented a table of nuclear sizes of non Mauthnerian cells on the two sides of the hind brain confirming the conclusions of Weiss and Rossetti insofar as stimulation of cell growth by thyroxin is concerned The cells on the side of the implant became significantly larger than those on the op posite side far removed from the source of the hormone The nuclei of Mauthner s cells on the side of the implant were reduced insignificantly ($P = 0\ 33$) compared with tho e of the opposite side However if the six specimens which had proceeded to stage XX with emergent fore limbs are selected from the nineteen that were treated the Mauthnerian nuclei on the side of the implant are then found to be significantly smaller than those on the opposite side (164 μ^2 versus 196 μ $P =$ 0 02) The conclusion was reached that involution of Mauthner s cells requires either high concentrations of thyroid hormone locally for sev eral days or high concentrations of the hormone locally in addition to earlier degeneration of lateral line nerve centers or tail motor centers as a result of a relatively high hormone concentration in the general circulation Apparently one or more of these conditions did not obtain in the thirteen animals which did not proceed to the stage of forelimb emergence Thus Mauthner s cell reacts by involution to the thyroid hormone while other small and intermediate sized cell types nearby react by growing as pointed out by Weiss and Rossetti

under the action of thyroxin (Weiss and Ferris, 1954*a*, *b*, *c* *d*) It is invaded very much like the invasion of germinal cords through the basement membrane of the ovary Nests of epidermal cells which penetrate the basement membrane form the subcutaneous glands The Leydig cells disappear Again there is a definite local effect on the basement membrane At this time the invasion of connective tissue cells into the microstructural lamellae of the basement membrane can be seen electronmicrographically, they enter along cleavage lines forming the dermis This can be done only by cells dissolving the ground substance that holds the membrane together Either these cells develop at this time a hyaluronidase or some other enzyme which becomes activated by thyroxin, which seems unlikely or the ground substance of the basement membrane becomes susceptible to the enzymatic action of these cells at this time, which seems more likely Otherwise they could not invade This links up with what was said earlier about the action of relaxin on the ground substances and perhaps even with the invasiveness of tumors cancers, etc There probably is a common denominator somewhere, and the behavior of the basement membrane of amphibian larvae in response to thyroxin may offer an unusually favorable point of attack for an understanding of the molecular mechanism of action of the thyroxin molecule WEISS considers metamorphosis of amphibians to be a gold mine for analyses of this type

KOLLROS recalled that Kollros and Kaltenbach (1952) reported observations on the local metamorphosis of the skin Thyroxin cholesterol pellets implanted under the skin produced neat patches of metamorphosed skin surrounded by larval skin He showed a hypophysectomized animal treated with thyroxin alone at a concentration of 0 6 μg/liter It had developed to stage XIII and possessed rather well developed glandular ridges Control animals at this stage have no trace of skin glands let alone patent glands He showed another animal which had been treated similarly but which had developed to stage XX—the stage of forelimb emergence—before fixation It exhibited a very marked junction between the skin of the tail base and the more dorsal skin which shows adult pigment spots and which will persist and the more ventral skin, which retains larval characteristics and pigmentation and which will disappear with the rest of the tail The distinction is much clearer than in the normal animal at stage XX In section glands and a stratum corneum can be seen in the dorsal skin the ventral skin lacks glands and retains the larval arrangement of layers of cells Incidentally the corneal reflex was elicitable from the treated animal for over 2 months, whereas normally this reflex is elicitable only 4 or 5 days prior

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